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Drinking Water Addendum to the Criteria Document for Trichloroacetic Acid

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Table of Contents

List of Tables

List of Figures

Chapter I. Executive Summary

This document is an addendum to the *Final Draft for the Drinking Water Criteria Document on Chlorinated Acids/Aldehydes/Ketones/Alcohols* (U.S. EPA, 1994) and provides an update for trichloroacetic acid (TCA). This addendum provides study descriptions for newer studies of TCA that have been published between 1994 and 2004, as well as a few key studies published prior to 1994. Brief summaries of the older literature are introduced as appropriate in this addendum as a means to put the newer data into perspective and for synthesis of the discussion on the derivation of the Health Advisory for TCA.

TCA is a hygroscopic crystal in pure form, miscible in water, and usually exists in the environment in aqueous solutions. The molecular weight of TCA is 163.4. Chlorinated acetic acids are formed during chlorination of water that contains organic matter, primarily humic and fulvic acids. Formation of chlorinated acetic acids is higher in the presence of humic acid fractions of water than in the presence of fulvic acid.

TCA is readily absorbed by the oral route in rats and by the dermal and oral routes in humans. Once absorbed, TCA is available for systemic distribution, based on the detection of TCA in blood after oral exposure. Tissue distribution appears to be time-dependent; following intravenous administration of radiolabeled TCA, the highest concentrations were in plasma followed by kidney, and liver for the first 3 hours following exposure. In contrast, radioactivity in the liver exceeded that in plasma at 24 hours following exposure, perhaps reflecting the slow rate of elimination from the liver. Intermediate levels of radioactivity were measured in other tissues and were lowest in fat.

TCA appears to bind to plasma proteins, which might be an important determinant of partitioning of TCA from the plasma to target tissues. In one study, the unbound fraction of TCA in plasma was 0.53, with a blood:plasma concentration ratio of 0.76, suggesting that most of the TCA distributed in the blood would be available for uptake and distribution to tissues. One *in vitro* study indicates that plasma binding capacity of TCA is ~24-fold greater in humans than in mice and 2.5-fold greater in humans than in rats. The binding capacity in rats was ~10-fold greater than in mice. This difference suggests that more TCA is available to interact with tissues in the mice than in rats and humans. Although these *in vitro* data should be interpreted cautiously, this difference in plasma binding may be one reason why TCA has been found to induce cancer in the mouse, but not the rat.

No studies were identified on the tissue distribution of TCA in humans, however, the appearance of TCA in blood and urine of humans orally exposed to chlorinated solvents or chloral hydrate indicates that it is present in the systemic circulation as a downstream metabolite. No studies investigating the toxicokinetics or degree of maternal-to-fetus or blood-to-breast milk transfer of TCA were located in the literature.

TCA is not readily metabolized, based on minimal first-pass metabolism in the liver following oral dosing, and limited amounts of radioactivity excreted in exhaled air, or present as non-extractable radiolabel in plasma and liver, following i.v administration of radiolabeled TCA. The enzymes involved in TCA metabolism have not been determined; some *in vitro* studies suggest that biotransformation is likely to be mediated by cytochrome P450's metabolic pathways.

The primary route of excretion of TCA is in the urine (57-84% of an administered dose is excreted after 24-48 hours), with exhalation of CO_2 and fecal excretion contributing to a lesser extent. In one study, the elimination half-life following a single TCA dose was approximately 8 hours in rats. The elimination of TCA from the blood appears to be considerably slower in humans than in rodents exposed to chlorinated solvents, suggesting that chronic exposure to high doses might result in an increase in the internal dose of TCA. However, data on the rates of TCA elimination are based on studies of trichloroethylene and its downstream metabolites, including TCA. Thus, species differences might be due to differences in the internal dose of the parent compound (resulting from differences in systemic absorption) and/or in the rate of formation of TCA. On the other hand, rapid urinary clearance was observed in humans who were dermally exposed to low doses of TCA by walking or swimming in chlorinated pool water for 30 minutes. It has been suggested that the potential for TCA bioaccumulation at environmentally relevant human exposures is likely to be limited.

No physiologically-based pharmacokinetic (PBPK) models have been developed for TCA alone (i.e., as parent compound). However, PBPK models for TCA and DCA (dichloroacetic acid) in B6C3F1 mice exposed to trichloroethylene via oral dosing (by gavage in corn oil) or inhalation have been developed by several investigators.

EPA's Information Collection Rule (ICR) database contains extensive information on concentrations of MCA and TCA in drinking-water systems, and how those concentrations vary with input-water characteristics and treatment methods. The database contains information from 6 quarterly samples from 7/97 to 12/98, from approximately 300 large systems covering roughly 500 plants. The mean concentrations of TCA were 3.28 and 13.25 µg/L in treated groundwater and surface water, respectively.

In addition to TCA concentrations in drinking water, there are some limited data on TCA concentrations in foods. TCA in foods can originate through processing using chlorine disinfectants and though cooking in water containing TCA. Air monitoring data are needed to evaluate whether inhalation exposure is a significant route of human exposure. Dermal absorption of TCA contributes less than 1% of total doses from routine household uses of drinking water. Although available data suggest that food and air may be significant sources of human exposure to TCA, these data are inadequate to quantify the contributions of each of these sources for an overall assessment of human exposure. Thus, the default relative source

contribution (RSC) from drinking water (i.e., 20%) is used to estimate lifetime health advisories for this chlorinated acetic acid.

In short-term oral toxicity studies with TCA, high doses of approximately 600 mg/kg/day resulted in decreased food consumption and body-weight loss. Alterations in intermediary carbohydrate metabolism (e.g., decreased lactate levels in several tissues) have also been observed. The liver has consistently been identified as a target organ for TCA toxicity in short-term and longer-term studies. Indicators of peroxisome proliferation have been the primary endpoints evaluated, with mice reported to be more sensitive than rats. In B6C3F1 mice exposed for 10 weeks to drinking water doses $\geq$125 mg/kg/day, TCA induced peroxisome proliferation (in the absence of effects on liver weight); the No-Observable-Adverse-Effects-Level (NOAEL) was 25 mg/kg/day. In F344 rats exposed to TCA in drinking water for up to 104 weeks, peroxisome proliferation was observed at 364 mg/kg/day, but not at 32.5 mg/kg/day. Increased liver weight and significant increases in hepatocyte proliferation have been observed in short-term studies in mice at doses as low as 100 mg/kg/day, but no increase in hepatocyte proliferation was noted in rats given TCA at similar doses. More clearly adverse liver-toxicity endpoints, including increased serum levels of liver enzymes (indicating leakage from cells) and/or histopathological evidence of necrosis, have been reported in rats, but generally only at high doses. For example, in the 2-year chronic drinking-water bioassay with F344 rats just described, increased hepatocyte necrosis was observed only at the highest dose tested, 364 mg/kg/day.

The potential reproductive toxicity of TCA has not been adequately tested. No animal studies were identified that evaluated this endpoint. The results of an *in vitro* fertilization assay indicated that TCA might have the potential to decrease fertilization. The available data suggest that TCA is a developmental toxicant at maternally toxic doses. In the presence of maternal toxicity, TCA increased resorptions, decreased implantations, and increased cardiovascular malformations at 291 mg/kg/day in one drinking water study, decreased fetal weight and length, and increased cardiovascular malformations at 330 mg/kg/day a gavage study and decreased fetal body weights at 300 mg/kg/day in another gavage study. None of these studies identified a NOAEL.

TCA was not mutagenic in the Ames assay in *Salmonella typhimurium* strain TA100 in the absence of metabolic activation. In modified Ames assays with *Salmonella typhimurium*, mixed results were reported. TCA was weakly mutagenic in a mouse lymphoma assay. Studies reporting the effect of TCA on DNA strand breaks have also yielded mixed results. A recent study found that chromosome damage is not induced by TCA at neutral pH; in contrast, another study showed evidence of TCA-induced clastogenicity (small colonies) in mouse lymphoma cells at neutral pH.

In carcinogenic gavage bioassays, TCA induces liver tumors in mice but not in rats. One mouse study showed an increased incidence of hepatic adenomas in female B6C3F1 mice at

drinking-water doses of 262 mg/kg/day and higher. In contrast, no increase in liver lesions was found in F344 rats given drinking-water doses up to 364 mg/kg/day. In addition, a variety of recent studies investigating epigenetic and genetic mechanisms of carcinogenicity have observed TCA-induced or TCA-promoted liver tumors in mice.

There are no epidemiology or clinical studies investigating the potential human health effects of TCA by any route of exposure. Human health-effects data for TCA are limited largely to case reports of accidental dermal poisonings and dermal injury caused by its use in chemical skin peeling applications and topical treatment of warts. TCA is corrosive to human skin and concentrated solutions (ranging from 16.9% to 50% TCA) have been used clinically in chemical skin-peeling treatments for many years. No studies investigating the toxicity of TCA in humans via the inhalation route were located.

TCA induces systemic, non-cancer effects in animals and humans that can be grouped into three categories: metabolic alterations, liver toxicity, and developmental toxicity. The primary site of TCA toxicity is the liver. It has been suggested that TCA disrupts regulation of pyruvate dehydrogenase activity, leading to altered carbohydrate metabolism, although the precise mechanisms are unknown. Other hypotheses include TCA-induced dysregulation of protein kinases that modulate glycogen-phosphorylase activity, resulting in TCA-induced glycogen accumulation in the liver. However, in a study with dichloroacetic acid (DCA), no alterations in glycogen-phosphorylase activity associated with glycogen accumulation were observed, and the authors suggested that TCA might also not be acting in this manner. Proposed alternative mechanisms include alterations in the molecular structure of glycogen leading to sequestration of the glycogen in a form that is difficult to mobilize, or changes in serum glucose or insulin levels resulting in glucose accumulation. Peroxisome proliferation, as indicated by changes in markers of peroxisomal proliferation such as cyanide-insensitive palmitoyl-CoA oxidase (PCO) and increased 12-hydroxylation of lauric acid, is thought to play a role in at least some of the observed liver effects induced by TCA. Although TCA induces developmental toxicity in rats at maternally toxic doses and in a number of *in vitro* test systems, the mechanism for the developmental toxicity is not known. Physiologically-based pharmacokinetic modeling has suggested that TCA behaving as a weak acid might induce developmental toxicity by changing the intracellular pH in the fetal/embryo compartment Alternately, peroxisome proliferation might be involved in TCA's developmental toxicity; however, the mode of action is unknown.

A variety of mechanisms have been suggested as contributing to TCA-induced liver tumorigenesis. Of these, peroxisome proliferation and altered regulation of cell growth have the most supporting data. There is little evidence for a role of direct genotoxicity of TCA itself, oxidative DNA damage, or regenerative hyperplasia. The role of peroxisome proliferation is unclear, in part because liver tumors are only induced in mice, and the peroxisome proliferative response is activated in both mice and rats. Further, humans have been reported to be less affected by exposure to peroxisomal proliferators than either mice or rats, and thus the relevance

of this mode of tumor induction to human carcinogenesis may be low or non-existent. A more convincing argument case can be made for altered regulation of cell growth and proliferation in subpopulations of cells, thus providing a selective growth advantage in chemically- or spontaneously-initiated cells.

The Health Advisory (HA) values for TCA are summarized in Table I-1. No suitable studies were identified for derivation of the One-Day HA. A NOAEL of 25 mg/kg/day for increased relative liver weight, accompanied by increases in indicators of peroxisomal proliferation in B6C3F1 mice given TCA in drinking water for 21 days was used to derive a Ten-Day HA of 3 mg/L (3000 μg/L) for a 10-kg child. This Ten-Day HA was used as a conservative value for the One-Day HA. A NOAEL of 36.5 mg/kg/day, based on liver histopathological changes observed in Sprague-Dawley rats given TCA in drinking water for 90 days, was used to derive a Longer-Term HA of 0.4 mg/L (400 μg/L) for a 10-kg child and 1 mg/L (1000 μg/L) for a 70-kg adult. A NOAEL of 32.5 mg/kg/day, based on liver histopathological changes in F344 rats exposed to TCA in drinking water for 2 years, was used to calculate a DWEL of 1 mg/L (1000 μg/L) and a Lifetime HA value of 0.02 mg/L (20 μg/L), assuming an RSC of 20%.

According to EPA's 1999 *Guidelines for Carcinogen Risk Assessment*, TCA is classified as having "*suggestive evidence of carcinogenicity, but not sufficient to assess human carcinogenic potential*." The evidence from animal data is suggestive of carcinogenicity, which raises a concern for carcinogenic effects, but is not sufficient for a conclusion regarding human carcinogenic potential.

Table I-1. Summary of Health Advisory Values for Trichloroacetic acid [(a)]

Chemical	One-Day HA	Ten-Day HA	Longer-Term HA		Lifetime HA
			Child	Adult	
TCA	3	3	0.4	1	0.02

[a]mg/L

Chapter II. Physical and Chemical Properties

Available data on the physical and chemical properties of trichloroacetic acid (TCA) are summarized in Table II-1. The data contained in Table II-1 apply to the pure form of the chemical, which usually exists in the environment in aqueous solution.

Table II-1. Physical and Chemical Properties of Trichloroacetic Acid[1]

Property	Trichloroacetic acid (TCA)
CAS Registry No.	76-03-9
Formula	Cl_3CCOOH
Molecular weight	163.39
Appearance/color/form	White hygroscopic rhombohedral crystals
Odor	sharp, pungent
Density (g/mL)	1.60-1.63
Melting point (°C)	58.0
Boiling point (°C)	197.5
Octanol-water partition coefficient (log P)	1.33-1.7
Vapor Pressure (mm Hg) at 25°C	0.16 (1.0 at 51°C)
(pK_a) at 25°C	0.512-0.70
Henry's Law constant at 25°C (atm-m^3/mole)	$2.4x\ 10^{-8}$
Solubility: Water Alcohol Ether	 1.3×10^7 mg/L soluble soluble

1. Adapted from U.S. EPA (1994), HSDB (2004), and CCOHS, 1996

Chapter III. Toxicokinetics

The following sections summarize the available data on the absorption distribution, metabolism and excretion of TCA. A number of the studies that are cited use radiolabeled compounds. When this is the case the position of the radiolabeled atom(s) will be specified if known.

A. Absorption

Older short-term studies with mongrel dogs (Hobara *et al.*, 1988a) and male B6C3F1 mice (Styles *et al.*, 1991) indicate that most of an orally administered dose of TCA is rapidly absorbed. TCA concentrations in the plasma or liver peak in the first hour following oral dosing. Similar observations are reported in the more recent studies summarized here.

Quantitative evidence for systemic absorption of TCA following oral dosing was provided in a toxicokinetic study by Schultz (1999). Male F344 rats were given single oral or intravenous (IV) doses of 500 μmol/kg (82 mg/kg) of TCA. Concentrations of the parent compound were monitored in blood at various times for up to 48 hours. Concentrations of the parent compound in the urine and feces were measured at 48 hours after dosing. Key results from this study are presented in Table III-1.

The oral bioavailability of the administered compound was determined from the ratio of the blood area-under-the-curve (AUC) for the oral and IV doses. Based on this measurement, the oral bioavailability was reported by the study authors as 116% for TCA. The fact that the oral bioavailability is high suggests that TCA is not extensively metabolized via first-pass metabolism. The AUC for oral dosing was slightly greater than that following IV dosing, but the degree of absorption cannot be greater by the oral route because IV dosing presumes 100% absorption. Thus, the reported oral bioavailability of 116% likely reflects measurement or statistical variability and/or differences in clearance rate by the two routes of administration.

As a measurement of absorption rate, the mean time-to-peak blood concentration was determined to be 1.55 hours following oral dosing. The mean absorption time, which was determined as the difference in the mean residence time in blood following dosing via oral and IV routes, was reported as 6 hours for TCA. The mean absorption time is dependent on clearance from the blood as well as the absorption rate; therefore, the longer mean absorption time as compared to time-to-peak blood concentration of 1.55 hours might also reflect slower clearance following oral dosing. Taken together, the data from this study show that TCA is readily absorbed following a single oral bolus dose.

Table III-1. Toxicokinetic Data for TCA in F344 Rats[a]

Parameters determined following IV dosing with 500 μmol/kg	**TCA (82 mg/kg)**
Area under blood concentration-time curve AUC (μM-h)	5406 ± 144[b]
Amount excreted in urine in 24 h (% Dose)	48.5 ± 13.0
Steady-state apparent volume of distribution (mL/kg)	782 ± 117
Total body clearance (mL/hr-kg)	92.5 ± 2.5
Renal clearance (mL/hr-kg)	42.1 ± 9.9
Mean residence time (hr)	8.5 ± 1.6
Elimination half-life (hr) - total time course	8.0 ± 2.4
Unbound fraction in plasma (f_u)	0.53 ± 0.04
Blood/plasma concentration ratio	0.76 ± 0.16
Parameters determined following oral dosing with 500 μmol/kg	**TCA (82 mg/kg)**
Area under blood concentration-time curve AUC (μM-hr)	6304 ± 1361
Maximum concentration in blood (μM)	340±17
Mean residence time (hr)	14.5 ± 4.7
Time to peak blood concentration (hr)	1.5 ± 0.3
Mean absorption time (hr)[c]	6.0
Oral Bioavailability (%)[d]	116[e]

[a]Adapted from Schultz, 1999
[b]Mean ± standard deviation
[c]Calculated as the difference between the mean residence time following IV versus oral dosing
[d]The ratio of the mean values for AUC for oral versus IV dosing x 100%
[e]This value likely reflects either measurement or statistical variability, and/or differences in clearance rate between oral and intravenous routes of administration, as oral bioavailability cannot actually be greater than 100%.

TCA also appears to be readily absorbed through the skin. Kim and Weisel (1998) investigated the potential for dermal absorption of TCA by evaluating the correlation between exposure to TCA (swimming pool water concentration × exposure duration) and urinary excretion of TCA in four human volunteers (two/sex). Swimming-pool-water concentrations were measured before and after volunteers either walked or swam in the pools for 30 minutes. TCA concentrations in the swimming pool water varied from 57 to 871 μg/L with a mean of 420 μg/L and a median of 278 μg/L. In one set of exposures, the four subjects simultaneously walked around in the pool (dermal exposure only), submerging the entire body exclusive of the head for 30 minutes. In another set of exposures, the same four subjects swam for 30 minutes (resulting in both dermal exposure and presumed oral exposure from incidental ingestion of pool water). Entire urine voids were collected for at least 24 hours before exposure, and 20-40 hours following exposure, at approximately 3-hour intervals. Additional urine samples were collected 5-10 minutes before and after exposures.

During the 24 hours prior to and following exposure, subjects avoided activities such as drinking chlorinated tap water or visiting the dry cleaners, which might result in urinary TCA excretion. Background levels of TCA were calculated from the amount of urinary TCA excreted in the urine void during the 3 hours prior to pool-water exposure. The amount of urinary TCA associated with exposure was estimated by subtracting background levels from TCA levels in the urine void collected 5 to 10 minutes following the exposure period. The results showed that urinary TCA levels were elevated in the 10-minute period following exposure, and generally returned to pre-exposure levels within 3 hours. Post-exposure urinary excretion of TCA was highly variable, ranging from approximately 1.1-fold to 3.9-fold greater than background excretion levels. Higher exposures resulted in higher amounts of urinary TCA adjusted to the subjects' body surface area, suggesting a dose-response relationship. The correlation coefficient for TCA exposure and amount excreted was 0.80 ($p= 0.00005$).

In another study by the same authors (Kim and Wiesel, 1998), one male and one female volunteer ingested 500 mL of chlorinated drinking water containing less than 10 μg/mL of TCA, and urine was collected for the following 24 hours. No increase in TCA levels were observed following ingestion, which the authors suggested was due either to variability in background excretion rates or to TCA not being excreted with urine within the time period that urine samples were collected. The rapid appearance of TCA in urine following dermal exposure in swimming-pool water suggested that dermal absorption of TCA was rapid. Skin permeability was not estimated for TCA.

In a more recent study by Xu *et al.* (2002), a permeability coefficient of $1.9 \times 10^{-3} \pm 5$ cm/hr for human skin was determined experimentally for TCA. Using dermal uptake methods recommended by EPA (U.S. EPA, 1992), the study authors estimated a total dermal uptake (showering and bathing) of 0.052 μg/day. This value is 0.3% of a total ingestion dose of 16.8 TCA μg/day (based on a water consumption rate of 1.4 L/day). These data indicate that dermal uptake of TCA in drinking water is unlikely to be significant compared to ingestion uptake.

These studies confirm earlier findings and demonstrate that TCA is readily absorbed by the oral and dermal routes. No new studies were identified on the degree or rate of TCA absorption following inhalation exposure.

B. Distribution

Oral gavage studies in rodents reveal that orally administered TCA is available for systemic distribution in the plasma of animals. Styles *et al.* (1991) reported that in male B6C3F1 mice administered a single oral dose of 500 mg/kg [2-^{14}C]TCA, 43% of the administered radioactivity was found in the liver after 24 hours. The study authors considered the apparent binding in liver tissue to be the result of the metabolism of TCA and subsequent incorporation of metabolites into cellular macromolecules. In another single dose gavage study, male F344 rats and B6C3F1 mice were administered 20 or 100 mg/kg (4/dose group) [^{14}C]TCA radiolabeled at both carbons (Larson and Bull, 1992). The majority of the radiolabel in the plasma was not associated with plasma protein, suggesting that most of the TCA distributed in the blood would be available for tissue uptake. TCA can also be recirculated systemically and has been reported to undergo cholecystohepatic circulation as well as reabsorption from the urinary bladder (Hobara *et al.*, 1987a; Hobara *et al.*, 1988b).

Numerous recent animal studies have been conducted to assess the distribution of TCA. Schultz (1999) administered male F344 rats a single oral or IV dose of 500 μmol/kg (82 mg/kg) of TCA and measured the parent compound in venous blood at various times for up to 48 hours. The fraction of TCA in plasma not bound to plasma protein (the unbound fraction) was estimated to be 0.53. The blood/plasma concentration ratio for TCA was 0.76, indicating some propensity for TCA to partition to the plasma, and was consistent with the ability of TCA to bind plasma proteins. Tissue concentrations were not measured in this study, but based on the similarity between the apparent volume of distribution and the total body-water volume in rats, TCA appeared to be widely distributed. The calculated steady-state apparent volume of distribution was 782 mL/kg for TCA, while the authors reported the total body-water volume for rats as approximately 660 mL/kg. Further evidence supporting wide tissue distribution of TCA in total body water is the low lipophilicity of TCA at physiological pH. The octanol-buffer partition coefficient (Log D) at pH 7.4 was reported to be -1.47 (Schultz, 1999). This negative value suggests that at physiological pH, TCA would have little propensity for accumulation in lipid-rich tissues and, thus, would likely be distributed in body water.

The dose-dependent partitioning of TCA between blood and liver was examined by Templin (1993). Male B6C3F1 mice were administered TCA via a single oral dose of 0.03, 0.12, or 0.61 mmol/kg (corresponding to 5, 20, or 100 mg/kg), and blood samples were taken at 1, 2, 4, 6, 9, 12, 18, and 24 hours following treatment. Four mice per treatment group were euthanized at each time point and all blood samples were analyzed separately. A pharmacokinetic analysis was conducted to determine the elimination rate constants, area under

the blood concentration time curve (AUC), and clearance values; TCA plasma protein binding was also assessed.

Based on both peak values and totals (AUC), TCA distribution favored the blood over the liver, and the partitioning of TCA into the blood increased with increasing dose of TCA in a nonlinear manner. Dosing with 0.03, 0.12, or 0.61 mmol/kg TCA resulted in peak blood concentrations of approximately 50, 250, or 475 nmol/mL, respectively, and peak concentrations of TCA in liver of approximately 50, 125, or 175 nmol/mL, respectively, as estimated from a figure presented in the paper. Partitioning to blood was also favored, based on AUC measurements. For example, the liver AUC to blood AUC ratio was approximately 0.75 for a peak TCA blood concentration of 50 nmol/mL, and approximately 0.45 for a peak blood concentration of 450 nmol/mL (estimated from a figure in the paper).

The degree of plasma protein binding was also concentration-dependent. The amount of TCA bound to plasma constituents was estimated *in vitro* by incubating [^{14}C]TCA with plasma, and determining the amount of unbound radioactivity and bound radioactivity (total radioactivity added minus unbound radioactivity). At TCA concentrations in plasma below 306 nmol/mL, approximately 50-57% of the TCA was bound to plasma constituents; at plasma concentrations above 306 nmol/mL, the percentage of TCA bound to plasma constituents decreased from 41% at an AUC of 306 nmol/mL to 23% for an AUC of 1,224 nmol/mL. The decrease in the percent of the bound TCA with increasing plasma concentration was consistent with the binding parameters for TCA estimated by Scatchard plot analysis of these *in vitro* data. The estimated K_D (the plasma concentration of TCA resulting in half-maximal binding) was 248 nmol/mL and the estimated B_{max} (the plasma concentration of TCA resulting in maximal binding) was 310 nmol/mL. Thus, plasma TCA concentrations of 306, 612, and 1224 nmol/mL equaled or exceeded the binding capacity of the plasma, and a lower percent of the TCA in the plasma was bound to plasma constituents.

Based on the determined binding parameters, oral doses of TCA between 20 and 100 mg/kg/day, which resulted in peak blood concentrations of 250 and 475 nmol/mL, respectively, would be expected to result in half-maximal to maximal plasma constituent binding in the mouse, and there would be more free TCA present at the higher dose. The concentration-dependent plasma binding is toxicologically significant because it determines the distribution of TCA from blood to target tissues. In addition, plasma binding would be expected to sequester free TCA and thus compete with TCA distribution to the tissues for metabolism. As plasma-binding capacity is saturated, more TCA becomes available for metabolism. The role plasma-protein binding plays in the distribution of TCA may be of significance to risk assessment because of potential species differences. In a recent review, Lash *et al.* (2000) noted that TCA is bound more efficiently to plasma proteins in the mouse than in humans, but quantitative differences were not presented.

Toxopeus and Frazier (1998) investigated the kinetics of TCA in isolated perfused rat liver (IPRL), using male F344 rats. The IPRL system was dosed with either 5 or 50 μmol of TCA, and TCA concentrations were monitored in perfusion medium and bile for 2 hours. Liver viability was assessed by measuring lactate dehydrogenase (LDH) leakage into perfusion medium and by the rate of bile production. At the end of the exposure period, the concentration of TCA in liver was measured.

In the study with 50 μmol TCA, the total TCA concentration (free and bound) in perfusion medium decreased slightly during the first 30 minutes and then remained constant for the duration of the exposure period; the total TCA concentration in the perfusion medium was relatively constant in the study with 5 μmol TCA. At the high dose, approximately 93% TCA was bound to albumin, and the free TCA concentration averaged 15.4 μM at 5 minutes of exposure and 14.9 μM at 120 minutes. At the low dose, 96% of the TCA was bound to protein, and the free TCA concentration was approximately constant at 0.9 to 1 μM over the study period. The calculated free TCA concentration in the liver intracellular space was higher than the free TCA concentration in the perfusion medium. Enzyme leakage and bile flow were similar at both TCA exposure levels to that in the control liver, indicating the absence of hepatotoxicity. The authors concluded that the binding of TCA to albumin in perfusion medium limits the uptake of TCA by the liver, and that TCA is virtually unmetabolized by the liver. These findings are consistent with those from *in vivo* mouse studies (e.g., Templin, 1993) demonstrating TCA binding to serum protein, and suggest that TCA kinetics are determined by plasma-protein binding.

Yu *et al.* (2000) studied the tissue distribution of TCA in male F344 rats injected IV with [1-^{14}C]TCA at doses of 0, 6.1, 61, or 306 μmol/kg (0, 1, 10, or 50 mg/kg). The ^{14}C in blood and tissues was measured at various time points for up to 24 hours post-injection and the concentration of TCA (as TCA equivalents) was determined. Following IV injection, the concentration of TCA in various tissues peaked rapidly. Overall kinetic behaviors were similar at all three doses. No TCA metabolites were measured in plasma, urine, or tissue samples. At early time points, the highest TCA concentrations were measured in plasma, followed by kidney, red blood cells (RBC), liver, skin, small intestine, large intestine, muscle and fat for all three doses; the relative order of these concentrations remained unchanged up to 3 hours following dosing. Thus, the initial distribution of TCA in tissues appears to be independent of dose, although at the high dose, some nonlinear behavior was noted.

At 24 hours following dosing, the distribution pattern was markedly different, reflecting plasma and tissue differences in terminal disappearance rate constants. Disappearance of TCA equivalents from RBC, muscle, and fat was similar to, or faster than, plasma at all doses; disappearance rate constants for kidney and skin were slightly lower than plasma, whereas liver, small intestine, and the large intestine demonstrated significantly slower elimination. The most notable difference at 24 hours post-exposure was between plasma and the liver, when the total concentration of TCA equivalents in liver greatly exceeded that in plasma, perhaps reflecting the

slower terminal elimination rate constant from liver compared to plasma due to biological incorporation of TCA metabolites into hepatic intracellular components.

To more fully explain the differences of kinetics of TCA in the plasma and liver, the authors studied the binding characteristics of TCA in plasma and liver. Based on *in vitro* experiments, the authors concluded that there is much stronger binding of TCA in the plasma than in the liver. However, the authors also noted that it was not possible at the present time to determine whether TCA or its metabolite(s) binds covalently with macromolecules or whether radiolabeled carbon derived from TCA is metabolically incorporated into macromolecules. In addition, based on the rate of formation of extractable and non-extractable radioactivity, only limited TCA metabolism was observed. As an alternative to tissue covalent binding or metabolic incorporation into liver cells to explain the slower elimination rate of TCA from liver as compared with plasma, the authors investigated the hepatic intracellular accumulation of TCA. They determined that TCA binding in the liver was negligible and that the concentration of unbound TCA in the intracellular space was significantly higher ($p < 0.05$) than the biliary concentration of free TCA. Because the difference could not be attributed to TCA binding in the liver, the authors concluded that the slower elimination rate of TCA in the liver results from TCA being transported into hepatic cells faster than it is transported out of these cells.

In agreement with the results of Yu *et al.* (2000), Abbas and Fisher (1997) had previously determined partition coefficient values for TCA in B6C3F1 mouse tissues by a centrifugation method. The tissue to blood partition coefficients were 1.18 for the liver, 0.88 for the muscle, 0.74 for the kidney, and 0.54 for the lung. These data support the conclusion that TCA distributes preferentially to the liver in rats and mice. In contrast to the partition coefficients determined for the mouse, the tissue:blood partition coefficients for humans were slightly different. Using PBPK models developed by Fisher and his colleagues and incorporating whole blood and plasma TCA measurements taken from human volunteers exposed to trichloroethylene, Fisher (2000) reported human tissue:blood partition coefficients of 0.66 for the liver, 0.66 for the kidney, 0.47 for the lung, and 0.52 for muscle. Thus, tissue:blood partitioning may differ among species. Based on a review of limited data from animal studies and *in vitro* assays, Lash *et al.* (2000) have proposed that there may also be species differences in plasma protein binding of TCA between humans and mice.

This theory is supported by recent results regarding plasma binding of TCA in mice, rats, and humans. Lumpkin *et al.* (2003) used *in vitro* equilibrium dialysis techniques to determine plasma binding of these three species with 13 different concentrations of TCA ranging from 0.06 to 6,130 μM (0.01 to 1,000 μg/mL). The quantitation limits were 0.12, 0.12, and 0.18 μM for human, rat, and mouse plasma, respectively. As the amount of TCA added increased, the percent bound TCA remained relatively constant until binding capacity was exhausted. The calculated percent bound approached zero at 3,065 μM TCA in mouse plasma and for all species at 6,130 μM as the amount of TCA in the bound state remained constant and the TCA added after saturation was all found in the unbound state.

Human plasma was reported to have the highest binding capacity over the entire concentration range with a maximum binding (86.8%) at the lowest testable TCA concentration (0.12 μM). The mean bound fraction (81.6%) remained stable over the entire measurable concentration range. Maximum binding values in the rat and mouse were 66.6% and 46.6%, respectively with quasi-steady state levels of 38.6% and 19.1%, respectively. Models indicated the number of binding sites per protein varied from 2.97 for humans to 0.17 for mice. Single saturation binding process models gave the best fit to the data for all species. The binding capacities were 709, 283, and 29 μM, in humans, rats, and mice, respectively, indicating that humans were ~24- and 2.5-fold better at binding TCA than rats and mice, respectively, and rats were ~10-fold better at binding TCA than mice. These data suggest that following exposure to TCA, mice will have much more free compound in the bloodstream available to interact with tissues than will rats or humans. This difference in bioavailable TCA could explain the difference in the liver carcinogenicity between the two rodent species and may also imply a lower susceptibility of humans to TCA-induced liver cancer. It also suggests the need for caution in using the dose-response relationship in mice to predict the tumor response in humans. However, these *in vitro* data may not represent *in vivo* conditions and therefore, should be interpreted with caution.

No additional studies were identified that might confirm the nature and extent of species differences in TCA distribution. Indirect evidence in humans, primarily from studies involving exposure to chlorinated solvents such as trichloroethylene, tetrachloroethylene, and 1,1,1-trichloroethane, suggests that TCA is widely distributed. TCA is a metabolite of trichloroethylene, and has been frequently measured in the urine or blood of humans exposed to trichloroethylene as a result of environmental contamination (Ziglio, 1981; Ziglio *et al.*, 1983; Vartiainen, 1993; Skender *et al.*, 1994; Bruning *et al.*, 1998) and in human volunteer studies (NIOSH, 1973; Brashear *et al.*, 1997; Fisher, 1998). TCA is also found in the blood and urine of humans without known chlorinated-solvent exposures (Hajimiragha *et al.*, 1986) and in individuals exposed to low concentrations of TCA in swimming pool and drinking water (Kim and Weisel, 1998; Froese *et al.*, 2002; Bader *et al.*, 2004). These studies demonstrate that TCA, whether it is absorbed from external sources or is formed as a downstream metabolite of other compounds, appears in the blood and urine and is thus available for systemic distribution in humans.

No studies investigating the toxicokinetics or degree of maternal-to-fetus or blood-to-breast milk transfer of TCA were located.

C. Metabolism

Larson and Bull (1992) reported the formation of CO_2, glyoxylic acid, oxalic acid, glycolic acid, and dichloroacetic acid (DCA) following oral administration of 20 or 100 mg/kg [^{14}C]TCA to rats and mice. The authors suggested that TCA was metabolized by a reductive dehalogenation mechanism to form DCA. Lipid peroxidation induced by TCA was given as

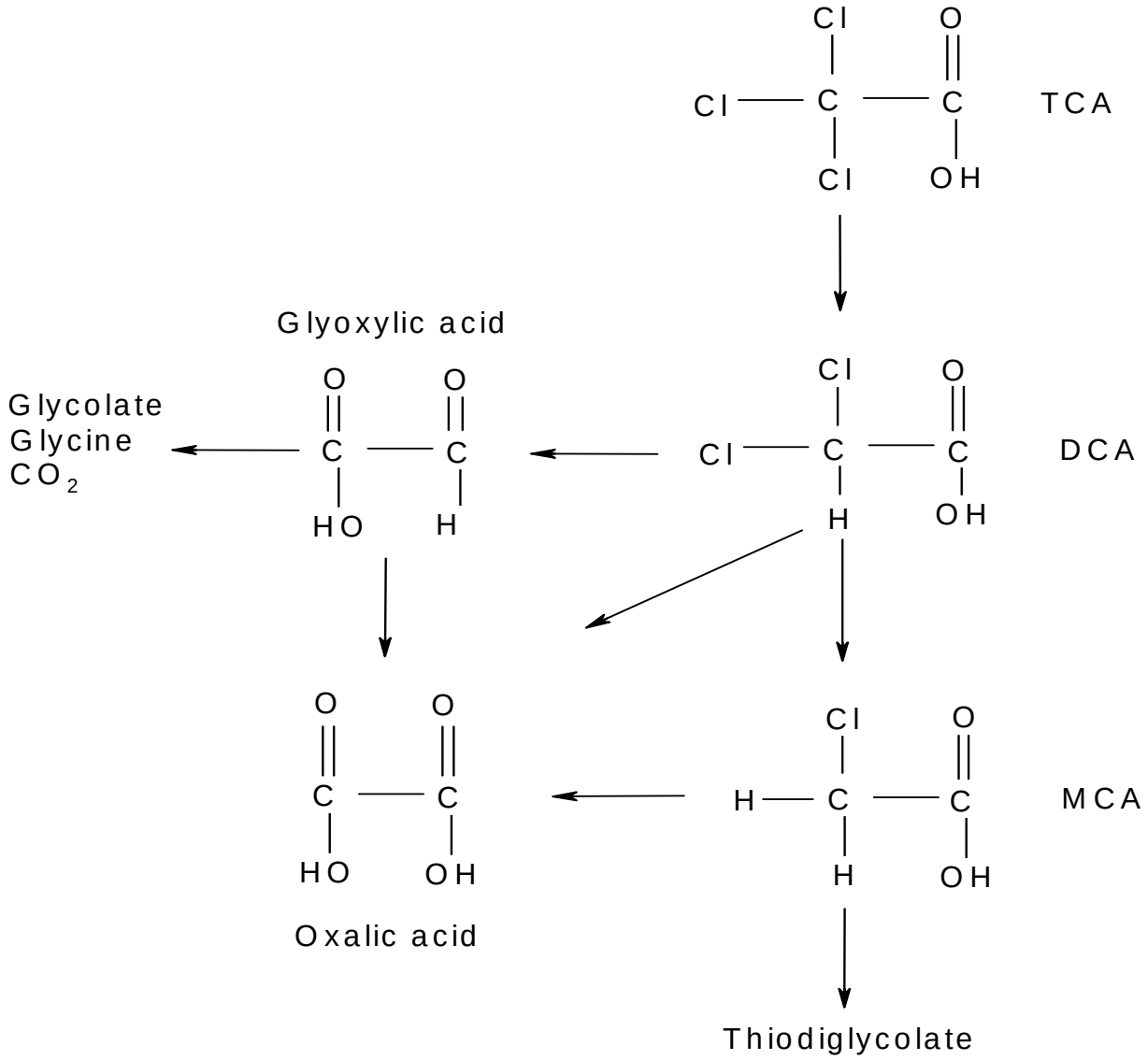
evidence for this mechanism, which would result in the formation of free-radical intermediates capable of binding to cellular lipids. The liver was suggested as the primary site of TCA metabolism, based on decreased TCA metabolism in mongrel dogs following hepatic by-pass (Hobara *et al.*, 1987b). Figure III-1 summarizes potential pathways for TCA metabolism.

The formation of both TCA and DCA as metabolites of trichloroethylene or chloral hydrate (a trichloroethylene metabolite upstream of TCA) suggested that DCA might be formed from TCA. In an attempt to address this possibility, Abbas *et al.* (1996) compared TCA and DCA kinetics following the administration of trichloroethylene or chloral hydrate. Male B6C3F1 mice were administered IV doses of chloral hydrate at 10, 100, or 300 mg/kg, and blood and urine samples were collected and examined for chloral hydrate (CH), trichloroethanol (TCOH), trichloroethanol glucuronide (TCOG), TCA, and DCA. The concentration of TCA gradually increased and approached a steady state over a 4-hour period. The blood AUC for TCA was 26.8, 368, and 818 μmol-hour/L at doses of 10, 100, and 300 mg/kg, respectively. Significant amounts of DCA were found in mouse blood, although at only a fraction (10-20%) of the TCA concentration. DCA remained in the systemic circulation over a 4-hour period and mimicked the shape of the TCA concentration-time curve. Based on unpublished data that the half-life of DCA in mice is only 12 minutes, the authors stated that the continued persistence of DCA in the presence of TCA suggested that DCA formation is dependent on TCA kinetics, implying that DCA is formed from TCA.

Recent evidence calls into question whether DCA is a metabolite of TCA, or at least the degree of conversion. Lash *et al.* (2000) discussed the evidence surrounding this controversy. According to this review, Larson and Bull (1992) may have over-reported DCA concentrations in male F344 and B6C3F1 mice, possibly due to the method used for DCA analysis. Ketcha *et al.* (1996, as cited in Lash, 2000) suggested that the analytical methods used in the earlier studies might have resulted in conversion of TCA in biological samples to DCA, and led to over-estimation of the formation of DCA. Based on these reports, Lash *et al.* (2000) concluded that the "true" concentrations of DCA in biological samples reported in these earlier animal studies are likely to be lower than the reported values, due to analytical artifacts. Thus, the degree (if any) of TCA metabolism to DCA remains unclear.

Schultz (1999) compared renal and blood clearance of TCA following a single-dose of 500 μmol/kg administered intravenously to male F344 rats and reported blood, renal, and nonrenal clearance rates of 92.5, 42.1, and 50.4 mL/hr-kg, respectively. Approximately 46% of the clearance of TCA from the blood was accounted for by renal clearance, and excretion of TCA in the feces was negligible. Therefore, as much as 54% of the removal of TCA from the blood could be accounted for by either metabolism or tissue distribution. No data were provided to determine the degree of metabolism in different tissues although the distribution to peripheral

Figure III-1. Proposed metabolism of TCA



Adapted from Bull, 2000 and Lash et al. 2000

tissues was assumed to be equivalent to that for the liver based on the volume of distribution at steady state. Since only the unbound portion in plasma is available to be removed by the kidneys and for uptake by the tissues, the low level of unbound TCA in plasma has an important impact on its toxicokinetics. Schultz et al. (1999) concluded that the rate of TCA metabolism was low in the F-344 rat.

TCA was poorly metabolized in F344 rats given IV injections of radiolabeled [1-^{14}C] TCA at doses of 0, 6.1, 61, or 306 μmol/kg (0, 1, 10, or 50 mg/kg) (Yu *et al.*, 2000). Although the fraction of the administered radioactivity excreted in the urine at 24 hours post-dosing was as much as 84% at the high dose, HPLC analyses of plasma, urine, and liver homogenate were unable to detect any of the reported metabolites of TCA (oxalate, DCA, glyoxalate or glycolate) indicating that the label was excreted as the parent compound). Nevertheless, about 8-12% of the radioactivity was eliminated in exhaled air as CO_2, indicating that some TCA was metabolized. Intravenous administration of TCA also resulted in a significant increase in non-extractable [1-^{14}C]-label in the liver and plasma. The non-extractable radiolabel was considered by the authors to represent metabolites bound to hepatic macromolecules which were either retained in the liver or secreted into plasma. Alternatively, the radiolabeled carbon may have been covalently bound to macromolecules in liver plasma. The amount of TCA metabolized in 24 hours, including excretion in exhaled air and non-extractable binding in the liver and plasma, was estimated to be less than 20% of the total administered dose.

Few data are available on enzyme pathways that might play a role in the metabolism of TCA. Pravacek and coworkers (1996) evaluated the hepatotoxicity of DCA and TCA on liver slices from male B6C3F1 mice, as well as the metabolic capacity of the liver for these two compounds. In the studies evaluating cytotoxicity (as evidenced by potassium content and liver enzyme leakage), the liver slices were exposed for up to 8 hours at concentrations of TCA ranging from 0 to 86 mM (0 to 14 μg/mL) TCA. To determine if TCA treatments can alter phase I or phase II biotransformations, the liver slices were exposed to a low or high concentration of DCA or TCA, and the conversion of 7-ethoxycoumarin to 7-hydroxycoumarin (a measure of phase I metabolism), and formation of sulfate and glucuronide conjugates of hydroxycoumarin (a measure of phase II metabolism) were assessed. TCA treatment with 1000 μg/mL increased phase I metabolism, but had no effect on phase II metabolism at either 25 or 1000 μg/mL. Metabolism of TCA was monitored by the rate of removal of the parent compound. The removal of TCA was not saturable at non-cytotoxic concentrations over the range of concentrations tested (0 to 5000 μg/mL); thus neither the K_m (the concentration at which half-maximal metabolic rate is reached) or V_{max} (maximum metabolic rate) was estimated.

Ni and coworkers (1996) studied the mechanism of TCA-induced hepatic toxicity in an *in vitro* system. Incubation of TCA with male B6C3F1 mouse-liver microsomes resulted in free-radical generation and lipid peroxidation. Lipid-peroxidation products that were observed included acetaldehyde, formaldehyde, malondialdehyde, acetone, and propionaldehyde. Incubation with liver microsomes from mice pretreated with pyrazole, a specific cytochrome

P450 2E1 (CYP2E1) enzyme inducer, induced about 2-fold higher lipid peroxidation. The authors also reported that in the same experimental system, the same molar concentration of chloral hydrate (CH) induced lipid peroxidation to the same extent as TCA, and the CH-induced lipid peroxidation could be inhibited by 2,4-dichloro-6-phenylphenoxyethylamine, a general cytochrome P450 inhibitor. Thus, the authors suggested that cytochrome P450 is the enzyme system responsible for metabolic activation of TCA, and that CYP2E1 might be the primary isoform responsible for this metabolism.

In order to determine if TCA-induced lipid peroxidation (see study summary in Chapter V) is due to the formation of radical intermediates following dehalogenation of TCA by cytochrome P450 enzymes, Austin (1995) evaluated the effects of pretreating mice with TCA. Male B6C3F1 mice were pretreated with 1000 mg/L (estimated to be 228 mg/kg/day by the study authors) TCA in drinking water for 14 days, then administered 300 mg/kg of TCA, DCA, or an equivalent volume of distilled water (control) by gavage as an acute challenge. Animals were sacrificed 9 hours following the acute challenge, and lipid peroxidation, peroxisome proliferation, and TCA-induced changes in phase I metabolism were measured. Measures of phase-I metabolism included (1) changes in 12-hydroxylation of lauric acid (an assay specific for CYP4A isoform activity, which is believed to be associated with induction of peroxisome proliferation in rats and mice (Gibson, 1989); (2) changes in p-nitrophenol hydroxylation (an assay specific for CYP2E1 activity); (3) immunoblot analysis for induction of cytochrome P450 isoforms CYP2E1, CYP4A, CYP1A1/2, CYP2B1/2, and CYP3A1; and (4) total liver P450.

Pretreatment with TCA increased 12-hydroxylation of lauric acid, demonstrating an increase in CYP4A activity (and apparently reflecting a peroxisome-proliferation response), whereas p-nitrophenol hydroxylation was unchanged, indicating no effect on CYP2E1 activity. Immunoblot analysis, a measure of the amount of protein, was consistent with the increase in CYP4A activity. Increased band intensities on the immunoblot appeared to occur at locations corresponding to those identified as the CYP4A2 and CYP4A3 isoform bands. Similarly, immunoblot analysis was consistent with the absence of an effect on CYP2E1 activity, and also showed no changes in CYP1A1/2, 2B1/2, and 3A1 protein levels. TCA pretreatment did not alter the overall amount of total liver microsomal P450.

These data demonstrate that pretreatment of mice with TCA modifies the lipoperoxidative responses (described in Chapter V) following acute challenge. The authors suggested that this results from activities associated with peroxisome proliferation and might be related to a shift in the expression of P450 isoforms. The increased levels of CYP4A in TCA-pretreated mice is consistent with results observed in other studies with other peroxisome proliferators (Okita and Okita, 1992).

Although the metabolism of TCA to DCA has been proposed (Larson and Bull, 1992), the degree to which this reaction occurs has been debated (Lash, 2000), and the mechanism of dehalogenation of TCA has not been conclusively determined. The metabolism of both TCA

and DCA to similar downstream metabolites, as described in this paragraph, suggests that they may be sequential metabolites in the same pathway.

In summary, the metabolism of TCA has not been well-characterized. While several studies have suggested that TCA is metabolized to DCA in mice (Larson and Bull, 1992; Abbas, 1996), concerns regarding potential over-estimation of DCA formation reduce confidence in these findings (Lash, 2000). TCA appears to be metabolized only to a limited extent in rats (Schultz, 1999; Toxopeus and Frazier, 1998; Yu *et al.*, 2000). Enzyme systems responsible for TCA metabolism have not been identified *in vivo*, but *in vitro* experiments with mouse tissues have provided limited evidence for involvement of a cytochrome P450-mediated pathway (Ni, 1996; Prevacek, 1996).

D. Excretion

No full toxicokinetics studies were identified for humans. However, TCA in urine is often measured as a biomarker for chlorinated-solvent exposure or exposure to disinfectant by-products as described previously in the distribution section. Froese *et al.* (2002) reported urinary half-lives of TCA in three of ten human volunteers drinking disinfected water from Australian drinking water systems to be 2.3, 2.9, or 3.7 days; confidence in the first two estimates was greater than that in the third. Ten samples taken over the course of the sampling period (Feb 1 to Mar 3) indicated that these individuals were exposed to mean TCA concentrations in drinking water that ranged from 1.8±0.5 μg/L to 29± 11 μg/L. The participants drank tap water for two weeks, TCA-free bottled water for two weeks, and tap water again for one week. Considerable intra- and interhuman variability in excretion of TCA was noted. The half-lives were comparable to others reported by the same researchers in a later study (Bader *et al.*, 2004). In this later investigation, five volunteers (three men, two women) drank tap water with TCA concentrations ranging from 50-180 μg/L for two weeks, then switched to TCA-free bottled water for two weeks. Urinary elimination rates were determined by measuring TCA concentrations in first morning urine (normalized to creatinine content). The following half-lives were determined (days): 2.1, 2.3, 2.5, 5.0, and 6.3. These studies indicate that urinary TCA is a viable biomarker of exposure, even in individuals who are exposed at the levels occurring in disinfected drinking water.

Rapid elimination kinetics of TCA were reported in humans following low-dose exposure to TCA from swimming pool water. Kim and Weisel (1998) reported rapid clearance of TCA following dermal-only or dermal-plus-oral exposures from swimming pool water (discussed earlier in Section III. A). TCA levels in the urine void collected 5 to 10 minutes after the 30-minute exposure in the pool were elevated and generally returned to pre-exposure levels within 3 hours. Post-exposure urinary excretion of TCA was 1.1- to 3.9-fold higher than background excretion levels, as estimated from TCA levels in urine voided during the 3 hours prior to pool-water exposures. Estimated dermal exposure to TCA (based on the product of exposure duration and TCA concentration in the pool water) was positively correlated with the urinary levels of

this compound. The authors suggested (Weisel, personal communication)[1] that, although other studies have reported a relatively slow elimination rate following oral or inhalation TCA exposures (Breimer *et al.*, 1974; Humbert *et al.*, 1994; Volkel *et al.*, 1998), the rapid elimination rate observed in the swimming pool study likely resulted from route-dependent and dose-dependent differences in TCA kinetics. TCA pool water concentrations were low, ranging from 57 to 871 μg/L, with a mean of 420 μg/L and a median of 278 μg/L. These dermal exposures resulted in doses on the order of 1 μg, compared with doses on the order of 1 mg/kg in the oral studies. This low dermal dose would be rapidly excreted by the kidneys before being available for uptake by the liver as occurs following oral dosing.

Blood elimination half-lives of TCA are fairly short in rodents. Blood elimination half-lives ranged from 5.4 to 6.0 hours for male B6C3F1 mice administered oral gavage doses of 0.03, 0.12, or 0.61 mmol/kg TCA (corresponding to 5, 20, and 100 mg/kg) (Templin *et al.*, 1993). Similar results were reported by Schultz *et al.* (1999), who reported that the elimination half-life was 8 hours for F344 rats after IV administration of 500 μmol/kg (82 mg/kg) of TCA.

The toxicokinetic studies in animals show that the major route of excretion of TCA is in the urine, with a minor amount exhaled as CO_2. Mice and rats given single oral doses of TCA exhibited similar patterns of excretion over 24 hours (mice) or 48 hours (rats) (Larson and Bull, 1992). Urinary excretion accounted for 57-72% of the administered dose, roughly 90% of which was eliminated as TCA. Other urinary metabolites identified included glyoxylic acid, oxalic acid, and glycolic acid. Exhalation of CO_2 accounted for 5-11% of the administered compound. However, concerns about the analytical methods used in this study limit confidence in the results. Experiments in mongrel dogs revealed that biliary excretion was minimal over periods up to 2 hours after IV administration of TCA (Hobara *et al.*, 1986).

More recent studies on the excretion of TCA have resulted in similar findings. Schultz (1999) measured parent compound concentrations in the blood, urine, and feces 24 hours after oral or IV dosing of male F344 rats with 500 μmol/kg TCA (82 mg/kg). Urine was the major contributor to blood clearance, while feces made a minimal contribution. Apparent renal clearance of the parent compound accounted for only 46% of the total clearance. (However, some of the apparent renal clearance was probably attributable to tissue binding.) Putative metabolites of TCA were not measured, and neither was the release of CO_2. Therefore, it is not possible to determine the contribution of each route of excretion to the total administered dose of the parent compound.

Yu *et al.* (2000) also reported that the major route of TCA excretion was the urine following IV injection of radiolabeled [1-^{14}C]TCA at doses of 0, 6.1, 61 or 306 μmol/kg (0, 1, 10, or 50 mg/kg) in male F344 rats. Within 9 hours post-injection, approximately 35-58% of the TCA-associated radioactivity was excreted in the urine at all three dose levels; at 24 hours post-

[1] C.P. Weisel, Robert Wood Johnson Medical School, Piscataway, NJ.

exposure, cumulative urinary excretion had increased to 47-84% (as estimated from a figure in the paper and confirmed by the senior study author[2]). Contributions of fecal and respiratory excretion to the total excretion were much lower. Within 24 hours post-injection, only 4-7% of the TCA-associated radioactivity was excreted in the feces, and about 8-12% was excreted in exhaled air. Urinary excretion was rapid and dose-dependent. At the low dose of 6.1 μmol/kg (1 mg/kg) TCA, the mean fraction of the initial dose excreted in the urine was 35% at 9 hours post-injection, and this percentage had increased to 47% at 24 hours following exposure. At the high dose of 306 μmol/kg (50 mg/kg) TCA, the fraction of the initial dose excreted in urine was reported as 58% at 9 hours post-injection and 84% at 24 hours. In contrast, the percentage of administered TCA eliminated via the feces and exhaled in the breath decreased with increasing dose. The terminal first-order rate constants for TCA disappearance from various tissues after administration of 6.1 μmol/kg (1 mg/kg) were determined. As measured by TCA-derived radioactivity, elimination from the liver, small intestine, and large intestine was slower than elimination from the plasma, RBC, muscle, and kidney.

Toxopeus and Frazier (1998) investigated the kinetics of TCA in isolated perfused rat liver (IPRL) test system, using livers from male F344 rats. The livers were perfused with either 5 or 50 μmol of TCA, and TCA concentrations were monitored in perfusion medium and bile for 2 hours. Uptake of TCA was limited, as discussed above in the Distribution Section. The total TCA concentration in bile remained relatively constant throughout the exposure period, averaging 44 μM. Bile excretion was linear over time and cumulative excretion was 0.1% of the total dose by the end of the experiment, suggesting that biliary excretion contributes minimally to overall elimination of TCA.

In summary, the existing data demonstrate that, in rodents, urine is the primary route of excretion of TCA, with exhalation of CO_2 and fecal excretion contributing to a much lesser extent (Hobara, 1986; Larson and Bull, 1992; Templin *et al.*, 1993; Schultz *et al.*, 1999; Yu *et al.*, 2000). The urine is also an important route of excretion in humans, although no quantitative data have been identified to estimate the relative contributions of other routes of excretion. Although human data are very limited, they suggest the possibility that human elimination rates might be route- and dose-dependent. Rapid elimination human kinetics were reported following low doses resulting from acute dermal absorption of TCA from swimming-pool water (Kim and Weisel, 1998).

E. Bioaccumulation and Retention

No new studies were identified that evaluated the bioaccumulation or retention of TCA following longer-term dosing by the oral, dermal, or inhalation routes. Based on the volume of distribution as steady state in F-344 rats it is unlikely that peripheral tissues will sequester or bioconcentrate TCA (Schultz *et al.*, 1999), however there may be binding of TCA to tissue

[2] John M. Frazier, Wright-Patterson Air Force Base, Ohio

macromolecules limiting the amount of free material available for metabolism or excretion. Rapid TCA clearance was observed following low-dose exposures by the dermal route (Kim and Weisel, 1998), suggesting a limited potential for bioaccumulation at doses likely to result from dermal exposure to TCA.

F. PBPK models

Abbas and Fisher (1997) developed PBPK models for TCA and DCA in B6C3F1 mice exposed to trichloroethylene through oral dosing (by gavage in corn oil), and these models were expanded by Greenberg *et al.* (1999) to include the inhalation route. The main trichloroethylene PBPK model was linked to five TCE metabolite sub-models for chloral hydrate, trichloroethanol, trichloroethanol glucuronide, DCA, and TCA. Each sub-model contained compartments for the liver, lung, kidney, and body. Abbas and Fisher (1997) experimentally determined the tissue:blood partition coefficients for all five TCE metabolites. The model was developed using literature values for V_{max} and K_m for trichloroethylene, literature values for physiological parameters, and the experimentally-determined tissue partition coefficients. Other parameters were fit using data for trichloroethylene and metabolites obtained from male B6C3F1 mice receiving a single gavage dose of 1200 mg/kg trichloroethylene. The model was validated using the other doses in the same study (300, 600, and 2000 mg/kg). Additional parameters for the inhalation model (Greenberg, 1999) were developed from male B6C3F1 mice exposed for 4 hours to 600 ppm trichloroethylene; the model was validated with data from a separate inhalation study conducted at 110-748 ppm trichloroethylene. The TCA model adequately described the TCA concentrations in the liver, lungs, kidneys, and blood following trichloroethylene exposure, as well as urinary excretion of TCA following oral and inhalation exposure to trichloroethylene. The DCA models, however, did not fit the experimental data as well as the TCA models.

Since the TCA and DCA observed in the model validation studies came either from trichloroethylene metabolism or from conversion of other trichloroethylene metabolites, the results of directly administering TCA or DCA could not be determined. In addition, the first-order metabolic rate constants for the conversion of TCA to DCA, and for conversion of DCA to other metabolites, were markedly different in the oral and inhalation models, suggesting that there might be route dependency in the metabolism of TCA and DCA. However, because the metabolic rate constants were estimated from TCA and DCA derived from oral versus inhalation exposure to trichloroethylene, the differences in TCA and DCA metabolic rate constants could simply be secondary to metabolic differences upstream of TCA.

Fisher (2000) developed a human PBPK model for trichloroethylene. To account for trichloroethylene metabolism and excretion, the model also included two sub-models, one for TCA and one for trichloroethanol. The sub-model for TCA was constructed to model concentrations of TCA in human blood and urine following inhalation exposure to trichloroethylene. These sub-models included compartments for lung, kidney, body, and liver. The model was optimized using sex-specific metabolic rate constants and partition coefficients

for humans exposed to 50 or 100 ppm trichloroethylene. Using the model, the authors successfully estimated TCA concentrations in the blood and urine in exposed males and females. As the TCA modeled in this study came from trichloroethylene metabolism, rather than a direct exposure to TCA, the usefulness of the model for making judgements about the toxicokinetics of TCA following direct exposures is limited.

G. Summary

TCA is readily absorbed by the oral route in rats (Schultz, 1999) and by the dermal and oral routes in humans (Kim and Weisel, 1998). Once absorbed, TCA is available for systemic distribution, based on the appearance of TCA in blood after oral exposure in rodents (Templin, 1993; Schultz, 1999). Tissue distribution of TCA appears to be dependent on the time of measurement following dosing. TCA appears to bind to plasma proteins (Lumpkin *et al.*, 2003; Templin, 1993; Toxopeus and Frazier; 1998; Schultz, 1999; Yu *et al.*, 2000), which is an important determinant of the extent to which TCA partitions from plasma into target tissues. No studies were identified that investigated the tissue distribution of TCA in humans. No studies investigating the toxicokinetics or degree of maternal-to-fetus or blood-to-breast milk transfer of TCA were located.

TCA is not readily metabolized, as indicated by minimal first-pass metabolism in the liver following oral dosing with TCA (Schultz, 1999) and by limited amounts of radioactivity excreted in exhaled air or present as non-extractable radioactivity in plasma and liver following IV administration of [1-^{14}C]TCA (Yu *et al.*, 2000). Some studies suggest that TCA is metabolized to DCA (Larson and Bull, 1992; Abbas, 1996). However, confidence in these results is decreased by concerns regarding potential over-estimation of DCA levels due to analytical artifacts (Lash, 2000). The enzymes involved in TCA metabolism have not been determined, but some *in vitro* studies suggest the involvement of cytochrome P450s (Ni, 1996; Pravecek, 1996).

The primary route of excretion of TCA is in the urine, with exhalation of CO_2 and fecal excretion contributing to a much lesser extent (U.S. EPA, 1994; Templin *et al.*, 1993; Schultz *et al.*, 1999; Yu *et al.*, 2000). Based on the volume of distribution at steady state in F344 rats it is unlikely that peripheral tissues will sequester or bioconcentrate TCA (Schultz *et al.*, 1999), however there may be binding of TCA to tissue macromolecules limiting the amount of free material available for metabolism or excretion.

Chapter IV. Human Exposure

The sources of exposure to TCA have not been fully characterized, but TCA has been detected in rainwater, drinking water, and food, and in ambient air. This compound has also been used in industry, pharmaceutical preparations, and in hospitals.

A. Drinking Water Exposure

TCA concentrations were measured in samples of disinfected drinking water collected under the Information Collection Rule (ICR; U.S. EPA, 2000a). A cross- section of public water systems across the United States were required to collect samples of treated water by the U.S. EPA and measure the levels of selected disinfection byproducts. The following sections will present TCA data from the ICR as well as similar information from public water systems published by other researchers.

A.1 National Occurrence Data for TCA

This section presents the data collected from the ICR databases from those surface- and ground-water systems serving at least 100,000 persons. This database includes information gathered for 18 months from July 1997 to December 1998.

Section A.1.1 describes the ICR data set and analysis techniques used to present the data for the plants that submitted data under the ICR. The data in Sections A.1 and A.2 were taken from the online version of the ICR database (U.S. EPA, 2000a), and the explanation of the methods used was taken from the Draft EPA Document on Stage 2 Occurrence and Exposure Assessment for Disinfectants and Disinfection Byproducts (D/DBPs) in Public Drinking Water (U.S. EPA, 2000b).

A.1.1 ICR Plants

The ICR generated plant-level sets of data that link water quality and treatment from source to tap, and aid in understanding the seasonal variability in these relationships. The database contains information from 18 monthly or 6 quarterly samples collected between 7/97 and 12/98 from approximately 300 large systems covering approximately 500 plants. These samples were tested for influent and finished water-quality parameters (e.g., TOC, temperature, pH, alkalinity), selected DBP levels, and disinfectant residuals. Samples were collected at several locations throughout the distribution system to cover the entire range of residence times during which DBPs can form in finished water. Over the 18-month period, between 1407 and 1457 samples were taken from approximately 300 plants with surface water as their source, and approximately 580 samples were taken from 123 plants with groundwater as their source. For more detailed information, such as sampling locations and frequencies, refer to the ICR Data Analysis Plan (U.S. EPA, 2000c).

A.1.2 Quarterly Distribution System Average and Highest Value for TCA

This section describes the approach employed for the analysis of observed data for water-quality parameters, and for TCA concentrations. All data are categorized according to the types of source water - surface or ground. Plants having both surface and groundwater sources (mixed) or that purchase water are included in the surface water category. Quarterly Distribution System Average and Highest Value for TCA are presented in Table IV-1. Data presented in the table have been taken from the ICR database as provided to avoid misrepresentation or misinterpretation. Therefore, although all data in the table are presented with two decimal points (as provided in the ICR database), this does not necessarily represent the actual precision of the data.

The quarterly distribution system average is an average of the following four distinct locations in the distribution system.

- Distribution System Equivalent (DSE) location;
- Average 1 (AVG 1) and Average 2 (AVG 2) locations: Two sample points in the distribution system representing the approximate average residence time as designated by the water system; and
- Distribution System Maximum: Sample point in the distribution system having the highest residence time (or approaching the longest time) as designated by the water system

The quarterly distribution system highest value is the highest of the four distribution system samples collected by a plant in a given quarter.

As shown in Table IV-1, the mean concentrations of TCA were consistently lower in treated groundwater than treated surface water. The non-detects were treated as zero for the calculation of the mean, median, standard deviation, p10 and p90 values for this and all subsequent evaluations of the data (U.S. EPA, 2000a). The lowest mean concentrations are associated with the highest percentage of non-detects. The mean concentrations of TCA (averaged across the four sampling locations) were 3.28 and 13.25 µg/L in treated groundwater and surface water, respectively.

Table IV-1. TCA Quarterly Distribution System Average and Highest Value[1]

Source	Quarterly Dist. Sys. Average	Plants	N	PctND %	Mean µg/L	Median µg/L	STD µg/L	Min µg/L	Max µg/L	p10 µg/L	p90 µg/L
SW	Average	304	1457	4.74	13.25	10.75	11.95	0.00	116.50	1.55	28.50
	High	304	1457	4.74	16.16	13.00	14.81	0.00	174.00	1.90	34.00
GW	Average	123	582	54.30	3.28	0.00	7.20	0.00	58.50	0.00	11.00
	High	123	582	54.30	4.77	0.00	9.82	0.00	80.00	0.00	15.00

[1] Non-detects are treated as zero.

Source: SW - Surface Water, GW - Groundwater

Quarterly Dist. Sys.: Quarterly Distribution System (DS) Samples.
Average - quarterly average of 4 locations in DS.
High - highest of 4 locations in DS.

Plants: Number of plants sampled

N: Number of samples

PctND: Percent samples non-detect (detection limits not provided)

Mean: Arithmetic mean of all samples

Median: Median value of all samples

STD: Standard deviation

Min: Minimum Value

Max: Maximum Value

p10: 10th percentile

p90: 90th percentile

A.2 Factors Affecting the Relative Concentrations of TCA in Drinking Water

Sections A.2.1 - A.2.4 contain investigational information and ICR data on the effects of disinfection chemicals, influent bromide concentration, influent total organic carbon (TOC) concentration, and seasonal shifts, respectively in TCA concentrations. In a number of cases, there is a considerable difference between the mean and median and the p90 and maximum concentration for a given data set, indicating a skewed distribution. The standards deviation are also often large in comparison to the mean. Accordingly, caution should be used when weighing the statistical findings for the binary comparisons of TCA levels with a source water or treatment factor.

A.2.1 Disinfection Treatment

Chlorination has been the predominant water-disinfection method in the United States. However, water utilities are considering a shift to alternative disinfectants. Therefore, there is a need to understand the occurrence of DBPs in drinking water and the factors that may influence their formation. Several published studies (Boorman *et al.*, 1999; Richardson, 1998; Lykins *et al.*, 1994; Jacangelo *et al*., 1989) reported on the formation of DBPs under different disinfection conditions.

In a review on drinking water disinfection byproducts, Boorman *et al*. (1999) compared the concentrations of different drinking water disinfection byproducts, including TCA, formed by chlorination, ozonation, chlorine dioxide, and chloramination. Most of the data were available for surface water systems that used chlorination. For the systems using chlorination, TCA had a median and maximum concentration of 11 and 85 µg/L, respectively. The principal products formed by chloramination were similar to those formed by chlorination; additional information was not provided. Ozonation followed by chlorination and chloramination produced many of the same byproducts as seen with chlorination, but at lower concentrations; concentrations for individual compounds were not provided. Chlorine dioxide formed oxidation by-products similar to those formed by ozonation; additional details were not provided.

Richardson (1998) compared the relative concentrations of DBPs in drinking water using different treatment methods, and found that chlorination produced the highest concentration of DBPs, including TCA. Chlorine dioxide and chloramine, when compared to chlorine, produced fewer chlorinated by-products, and lower concentrations of these by-products. TCA was not produced by chlorine dioxide in measurable quantities. Compared to chlorine treatment, chloramine produced lower levels of chlorinated by-products, including TCA. The levels of DBPs, including TCA, were lower when ozone was the primary disinfectant (ozone followed by either chlorine or chloramine) than when chlorine or chloramine were used solely.

Lykins *et al*. (1994) investigated the formation of halogenated DBPs in the water distribution system, by predisinfecting and postdisinfecting the water with either chlorine or chloramine and holding the water for five days. They found that the use of chlorine produced the highest concentration of halogenated DBPs and that the concentrations could be reduced by adding ozone as a predisinfectant with postchlorination. Lykins *et al*. (1994) found that the highest concentration of TCA (62 µg/L) was formed when chlorine was the sole treatment method. The next highest concentration (25 µg/L TCA) was observed with ozone treatment followed by chlorine dioxide. Ozone treatment followed by chloramine resulted in 0.7 µg/L TCA, while chloramine treatment alone resulted in 1.7 µg/L TCA.

Jacangelo *et al*. (1989) examined the impact of ozonation on the formation and control of selected DBPs in drinking water at four utilities. Treatment modifications were made on the process train at each full or pilot-scale plant to incorporate ozone in the treatment process. For

two of the utilities, only total haloacetic acids (HAAs) were measured (Jacangelo *et al*., 1989), and no measurements were made of individual HAAs. The disinfection scheme that employed ozonation followed by chloramination resulted in large reduction in total HAAs, although the sample size did not allow for statistical analysis of the data.

For two utilities that measured individual HAAs, preozonation followed by chlorination decreased the total HAAs by 14 - 50%, but the magnitude and direction of change varied with the specific HAAs. For example, levels of TCA were 1.3 µg/L when chlorination was combined with ozonation, compared to 7.4 µg/L with chlorination alone. By contrast, at another utility the concentration for the combination of ozone and chlorine was 13 µg/L while that for chlorine alone was 22 µg/L.

Shifts to higher concentrations of dibromoacetic acid were observed when chlorine was used as the final disinfectant following ozonation, compared with chlorine only (Jacangelo *et al.*, 1989). The authors suggested that ozone reacts with bromide ions in the source water, resulting in the formation of hypobromous acid. Reaction of hypobromous acid and natural organic matter can produce brominated HAAs. When preozonation and postchlorination are practiced, competition exists between hypochlorous acid and hypobromous acid for organic matter, leading to varying concentrations of chlorinated and brominated HAAs (Jacangelo *et al.*, 1989).

Miltner *et al.* (1990) studied DBP formation and control in three surface water pilot plants employing three different disinfectant methods (chlorine, ozone followed by chlorine, and ozone followed by chloramine). On examination of the data using the Student's t-test, the authors found that the amount of TCA measured in finished water and in simulated distribution waters was lower ($p=0.05$) when ozonation was combined with chlorination or with chloramination than when chlorination was used alone.

A.2.1.1 Disinfection Treatment in ICR Database

Data on the concentrations of TCA were gathered from plants using several disinfection treatments. Those chemical disinfection treatments most commonly used (those affecting 10% or more of the plants evaluated), along with the ozonation treatments, are presented in Table IV-2 for TCA, respectively.

An examination of the ICR data for surface water plants using the Student's t-test indicates that ozone in the water-treatment plant and free chlorine or free chloramine in the distribution system may result in a significant reduction in the formation of TCA ($p = 0.05$) compared to that seen when free chlorine was used solely, as in the common (non-ozonation) chemical-disinfection processes. There were no significant differences in the mean concentrations of TCA among the common disinfection methods (non-ozonation) in in cases where the source water was groundwater (Table IV-2). There were no significant differences

Table IV-2. TCA by Disinfection Method (Quarterly Distribution System Average)[1]

Source	Disinfection Chemicals	Plants	N	PctND %	Mean µg/L	Median µg/L	STD µg/L	Min µg/L	Max µg/L	p10 µg/L	p90 µg/L
SW	Cl_2/Cl_2	178	805	2.11	14.85	12.75	10.92	0.00	80.88	3.53	29.95
	Cl_2_CLM/CLM	66	305	2.95	13.23	9.08	13.58	0.00	116.50	1.80	28.50
	O_3/Cl_2	7	25	0.00	5.58	5.13	3.28	0.33	16.78	2.40	9.03
	O_3/CLM	10	49	32.65	3.82	1.43	5.29	0.00	20.50	0.00	13.53
GW	$/Cl_2$	67	301	68.44	2.74	0.00	7.77	0.00	58.50	0.00	9.00
	Cl_2/Cl_2	39	169	53.85	2.22	0.00	4.96	0.00	36.25	0.00	6.75
	O_3/CLM	1	6	0.00	4.56	4.45	0.49	3.95	5.30	3.95	5.30

[1] Non-detects are treated as zero.

Source: SW - Surface Water, GW - Groundwater

$/Cl_2$: No disinfectant in Water Treatment Plant (WTP) and free chlorine in Distribution System (DS)

Cl_2/Cl_2: Free chlorine in WTP and DS

Cl_2_CLM/CLM: Free chlorine followed by chloramine in WTP and chloramine in DS

O_3/Cl_2: Ozone in WTP and free chlorine in DS

O_3/CLM: Ozone in WTP and chloramine in DS

Plants: Number of plants sampled

N: Number of samples

PctND: Percent samples non-detect (detection limits not provided)

Mean: Arithmetic mean of all samples

Median: Median value of all samples

STD: Standard deviation

Min: Minimum Value

Max: Maximum Value

p10: 10th percentile

p90: 90th percentile

from surface water. Although it appears that the concentrations of TCA were higher in the single plant in which groundwater was treated with ozone/chloramine than in plants using common disinfection treatment methods, statistical analysis cannot be conducted because of insufficient representative samples for comparison. In all cases the standard deviations for the samples were large relative to the mean, suggesting considerable variability among the samples. Comparison of the p90 values and the maximum detected concentration are also indicative of a skewed data set.

A.2.2 Bromide Concentration

Pourmoghaddas *et al*. (1993) examined the effects of source water and treatment characteristics, such as pH, reaction time, chlorine dosage, and bromide ion concentration, on the formation of HAAs. The study quantified nine HAA species in the presence of bromide ion at low, neutral, and high pH over time at two chlorine dosages. This study found a shift in the distribution of HAAs from chlorinated to brominated and mixed (bromochlorinated) halogenated species with increased bromide ion concentration.

TCA formation was not affected by changes in pH. Under all pH conditions and reaction times, the concentrations of TCA decreased rapidly with the incremental addition of bromide ion. Increasing reaction time resulted in increased TCA formation under all pH and bromide ion concentrations (Pourmoghaddas *et al*., 1993).

A.2.2.1 Bromide Concentration in the ICR Database

Table IV-3 presents the formation of TCA, respectively, as a function of influent bromide concentrations. Bromide concentrations tended to be lower in plants using surface water as a source than in those using groundwater as a source. For example, approximately 113 of the 293 plants using surface water as the source (39%) had influent bromide levels below the minimal reporting limit (MRL) of 20 ppb, while only 13 of the 123 plants using groundwater as the source (11%) had influent bromide levels below the MRL. Regression analysis of the ICR data indicates that there is no significant correlation ($\alpha = 0.05$) between influent bromide concentration and the mean concentrations of TCA in treated surface water or groundwater (Table IV-3). However, the standard deviations are large relative to the mean value indicating considerable variability in the data and lowering the confidence in the analysis.

In the case for disinfected surface water, the TCA concentration decreased when the bromide concentration was $\geq$100 ppb. This is consistent with the concept that increasing bromide concentrations lower the chlorinated DBPs because of increased formation of bromine-containing compounds. For treated ground water, distributions appear to be highly skewed by a few samples with high TCA concentrations because the median values for all bromide levels lower than 100 ppb are zero. It is only with the influent bromide concentration $\geq$100ppb that the median TCA concentration is above the detection level.

Table IV-3. TCA by Influent Bromide Concentration (Quarterly Distribution System Average)[1]

Source	Influent Bromide Conc. (ppb)	Plants	N	PctND %	Mean µg/L	Median µg/L	STD µg/L	Min µg/L	Max µg/L	p10 µg/L	p90 µg/L
SW	<MRL (20)	114	548	2.74	15.55	13.25	11.66	0.00	114.00	4.53	29.95
	20 - <30	41	199	1.01	13.57	9.00	13.70	0.00	116.50	2.35	29.60
	30 - <50	47	222	5.86	14.69	12.60	12.96	0.00	80.88	1.23	31.75
	50 - <100	59	276	4.71	12.67	9.51	11.30	0.00	59.53	1.73	27.05
	≥ 100	39	193	12.95	6.23	3.08	7.29	0.00	48.23	0.00	15.50
GW	<MRL (20)	13	65	69.23	1.08	0.00	2.69	0.00	12.00	0.00	2.98
	20 - <30	11	50	58	1.91	0.00	3.07	0.00	9.05	0.00	7.51
	30 - <50	26	109	58.72	5.70	0.00	11.17	0.00	47.10	0.00	23.00
	50 - <100	32	150	61.33	2.26	0.00	6.55	0.00	58.50	0.00	5.93
	≥ 100	41	208	41.35	3.77	1.26	6.24	0.00	35.18	0.00	11.25

[1] Non-detects are treated as zero.

Source: SW - Surface Water, GW - Groundwater
MRL: Minimum reporting limit
Plants: Number of plants sampled
N: Number of samples
PctND: Percent samples non-detect (detection limits not provided)
Mean: Arithmetic mean of all samples
Median: Median value of all samples
STD: Standard deviation
Min: Minimum Value
Max: Maximum Value
p10: 10th percentile
p90: 90th percentile

A.2.3 Total Organic Carbon (TOC)

Many researchers have documented that chlorine reacts with natural organic matter in water to produce a variety of DBPs, including trihalomethanes and haloacetic acids (Reckhow and Singer, 1990; Reckhow *et al*., 1990; Marhaba and Van, 2000). Natural organic matter in source water is generally monitored as total organic carbon (TOC). Arora *et al*. (1997) analyzed results of a DBP survey and a two-year DBP-monitoring study of more than 100 treatment plants of the American Water System from 1989 to 1991, and reported no correlation between raw-water TOC and the total of 5 haloacetic acid concentrations (HAA5: monochloroacetic acid, dichloroacetic acid, TCA, monobromoacetic acid, and dibromoacetic acid), in finished and distributed water samples. A significant correlation ($p < 0.01$) was found between TOC and HAA5 in plant effluent and distributed-water samples. However, only 11 and 15 percent of the variation in HAA5 was explained by TOC for the distributed-water samples and plant effluent, respectively.

A.2.3.1 TOC Concentration in the ICR Database

Table IV-4 presents data from the ICR database (U.S. EPA, 2000a) for the concentration of TCA, as a function of influent TOC concentrations. As shown in the table, influent TOC levels are higher, on average, in treated surface water than in treated groundwater. For example, 83 of the 123 plants that use groundwater as the source (67%) had TOC concentrations < 1 ppb, while only 12 of the 293 plants with surface water as the source (4%) had such low TOC levels in the influent water. Higher TOC levels in influent surface water are reasonable, as surface-water sources can contain decaying vegetation or animal matter, which would usually not be found in groundwater. Higher concentrations of TCA in treated surface water than in treated groundwater (see Table IV-1) may be attributable largely to these markedly higher TOC concentrations in surface water. Differences in the nature of the TOC material and the levels of disinfectant used by surface water systems compared to groundwater systems could also have been factors leading to the higher DCA levels in the surface water systems.

A regression analysis of the ICR data indicates that there is no significant correlation ($\alpha = 0.05$) between influent TOC concentration and the mean concentration of TCA in treated surface or groundwater (Table IV-4). There were only three samples for the ground water systems with TOC levels of 3-4 ppb, thus limiting the confidence in the mean and median for this data set.

Table IV-4. TCA by Influent Total Organic Carbon (TOC) Concentration (Quarterly Distribution System Average)[1]

Source	Influent TOC Conc. (ppb)	Plants	N	PctND %	Mean µg/L	Median µg/L	STD µg/L	Min µg/L	Max µg/L	p10 µg/L	p90 µg/L
SW	<1	12	61	24.59	8.16	6.48	7.95	0.00	28.75	0.00	19.50
	1 - <2	58	269	0.37	11.88	10.63	7.94	0.00	37.93	3.45	24.25
	2 - <3	99	477	4.40	12.03	9.48	9.90	0.00	67.03	2.35	23.28
	3 - <4	60	301	3.99	15.80	13.75	15.10	0.00	116.50	1.33	33.95
	≥4	69	322	5.90	14.91	12.78	14.04	0.00	114.00	1.30	32.50
GW	<1	83	405	70.86	1.06	0.00	4.08	0.00	58.50	0.00	2.15
	1 - <2	13	53	13.21	6.85	5.05	5.38	0.00	21.23	0.00	14.68
	2 - <3	8	36	50.00	4.18	0.26	8.26	0.00	36.25	0.00	19.25
	3 - <4	3	8	0	36.03	36.64	6.68	25.00	47.10	25.00	47.10
	≥4	16	80	5.00	8.50	4.63	8.52	0.00	35.18	1.38	19.90

[1] Non-detects are treated as zero.

Source: SW - Surface Water, GW - Groundwater
Plants: Number of plants sampled
N: Number of samples
PctND: Percent samples non-detect (detection limits not provided)
Mean: Arithmetic mean of all samples
Median: Median value of all samples
STD: Standard deviation
Min: Minimum Value
Max: Maximum Value
p10: 10th percentile
p90: 90th percentile

A.2.4 Seasonal Shifts

Williams *et al*. (1998) examined the concentrations of DBPs in winter and summer in raw intake water, finished water, and water within the distribution system main line at water treatment plants that used different disinfectant treatment combinations.

In the first survey, Williams *et al*. (1998) sampled raw-water intake, finished water (water after treatment prior to distribution), and waters near the midpoint of the distribution system for 52 Canadian water-treatment facilities. The raw-water sources included 28 rivers, eight lakes, three wells, a dammed impoundment, and two sources that were a mixture of the aforementioned sources. Pre- and/or post-chlorination (chlorine - chlorine) was used at 35 facilities and pre-chlorination coupled with post-chloramination (chlorine - chloramine) was used at ten facilities. Seven facilities used ozone coupled with chlorine or chloramine (ozone - chloramine). DBPs in the raw-water samples were either not present or were detected at very low levels.

In general, the mean concentrations of TCA were higher in the summer than in the winter, consistent with the observation that the formation rates of haloacetic acids increase with temperature (WHO, 2000). In facilities that used ozone coupled with chlorine or chloramine, as well as those that used pre-chlorination coupled with post-chloramination, TCA concentrations in the distribution system were comparable to those in the finished waters.

To better understand the seasonal and distribution system effects (effects of reaction time) on DBPs, Williams *et al*. (1998) conducted a second survey in which they sampled waters at five locations within the supply system once a month for one year at three facilities that used different treatment combinations (pre- and post-chlorination, pre-chlorination coupled with post-chloramination, and ozone coupled with chlorine). The data for each water plant consisted of DBPs in raw water entering the treatment system, finished water prior to entering the distribution system, and water at three points within the distribution system (D1 - closest to the plant, D3 - the end of the system, and D2 - a point midway between D1 and D3). Because there is increased reaction time with increasing distance from the treatment plant, the effects of reaction time may be evaluated by sampling water throughout the distribution system.

As in the previous survey, DBPs in the raw-water samples were either not present or detected at very low levels. TCA concentrations showed a seasonal trend, with concentrations higher in the summer months for the pre- and postchlorination and ozone - chlorine treatment scenarios for most sampling locations. The prechlorination followed by postchloramination treatment showed few differences among the seasons at all sampling points. For the pre- and postchlorination, and the prechlorination followed by postchloramination, the concentrations of TCA during all seasons increased with distance from the treatment facility until the end of the distribution system, when levels dropped. At the facility that used ozone coupled with chlorine, the concentrations of TCA in summer followed a similar trend. However, during the fall, winter

and spring months, the TCA concentrations in water from this facility increased continuously with distance from the facility to the end of the line.

A.2.4.1 Seasonal Shifts in the ICR Database

The seasonal mean concentrations of TCA are presented in Table IV-5. Examination of the data between the seasons using the Student's t-test indicates that there were no significant differences ($p = 0.05$) between the mean seasonal concentrations of TCA in treated groundwater nor were there any consistently significant differences ($p = 0.05$) between the mean seasonal concentrations of the chemical in treated surface water. In general, TCA levels were higher in the summer than in the winter for treated surface water.

B. Ambient Water

TCA may be present in source waters as well as drinking water. Effluent from waste water treatment plants where chlorine is used as a disinfectant can be a source of haloacetic acids in ambient surface water. In addition, discharges from paper and pulp mills which use agents containing chlorine for bleaching are another source of TCA in the environment (Juuti and Hoeskstra, 1998) along with that from any other industry relying on the disinfecting or whitening properties of chlorine.

C. Exposure to Sources Other Than Water

According to the HSDB Online Database (2004), TCA has been used in industry, pharmaceutical preparations, and in hospitals. TCA is used medically as a peeling agent for damaged skin, cervical dysplasia, wart removal, and removal of tattoos. It is also used in the manufacture of synthetic medicinal products and various organic compounds. It is employed as an etching and pickling agent for the surface treatment of metals, as a swelling agent and solvent in the plastics industry, as an auxiliary in textile finishing, and as an additive in mineral lubricating oils. Additionally, TCA also is used in the laboratory as a reagent to precipitate proteins and to detect various chemicals.

Between 1981 and 1983, The National Institute of Occupational Safety and Health (NIOSH) conducted a survey of a sample of 4490 businesses employing nearly 1,800,000 workers (NIOSH, 1990). Potential exposure estimates included surveyor observations of the use of TCA and trade-name products known to contain TCA. Exposure levels and routes of exposure were not reported in this survey, and more recent information on numbers of workers exposed is not available.

Table IV-5. TCA by Sample Quarter (Quarterly Distribution System Average) [1]

Sample Quarter	TCA					
	Surface Water			Ground Water		
	N	Mean (µg/L)	STD (µg/L)	N	Mean (µg/L)	STD (µg/L)
Summer '97	239	13.09	11.65	93	4.32	9.79
Fall '97	249	11.67	9.04	87	2.64	6.99
Winter '98	235	12.71	13.74	103	3.48	7.81
Spring '98	257	16.08	13.86	104	3.28	6.75
Summer '98	248	13.93	12.01	103	3.08	5.39
Fall '98	229	11.75	10.07	92	2.85	5.80

[1] Non-detects are treated as zero.

N: Number of samples

STD: Standard deviation

Sample Quarter:

Summer '97: July, August, and September

Fall '97: October, November, and December

Winter '98: January, February, and March

Spring '98: April, May, and June

Summer '98: July, August, and September

Fall '98: October, November, and December

During the period from 1981 to 1983, 35,124 workers were potentially exposed to TCA. The largest number of exposures (14,337) occurred in 735 general medical and surgical hospitals. Oil and gas field services (8072 exposures in 323 plants), medicinals and botanicals (4354 exposures in 21 facilities), medical laboratories (3989 exposures in 380 laboratories), and commercial testing laboratories (3534 exposures in 24 laboratories) made up the next largest numbers of potential exposures. The remainder of potential exposures, in decreasing numbers, included workers working with sausages and other prepared meats (333 in 48 plants), in pharmaceutical preparations (225 in 19 facilities), with instruments to measure electricity (174 in 10 plants), and with telephone and telegraph apparatus (106 in 3 plants).

C.1 Dietary Intake

In the *Final Draft for the Drinking Water Criteria Document on Chlorinated Acids/Aldehydes/Ketones/Alcohols* (U.S. EPA, 1994), chlorine was reported to be used in food production and processing, such as in disinfection of chicken in poultry plants; processing seafoods, poultry and red meats; sanitizing equipment and containers; cooling heat-sterilized foods; oxidizing and bleaching in the flour industry. The use of chlorine as a sanitizer is permitted by the FDA under 21 CFR 178.1010 at a maximum concentration of 100 ppm. Sodium hypochlorite is approved as a secondary direct additive for the washing of fruits and vegetables (21 CFR 173.315) and both sodium and calcium hypochlorite can be used for bleaching of modified food starch (21 CFR 172.892) at a concentrations of 0.0082 lbs chlorine equivalent/pound of starch. Therefore, TCA is likely be found as disinfection byproducts in a variety of food products.

In a study that investigated the uptake of several chemicals from soil by plants in a closed aerated laboratory soil-plant system (Schroll *et al*., 1994), TCA was taken up by the roots, and by the leaves via uptake from the air. Under both uptake scenarios, TCA was predominantly concentrated in the shoots of the plant. Bioconcentration factors for TCA were high, and ranged from 518 to 970 when taken up by both the roots and leaves.

Sutinen *et al*. (1995) found that under experimental conditions, TCA was taken into the needles of Scots pine seedlings via both roots and through the needle surface, through a simulated wet deposition fog. However, most of the TCA via the atmospheric route was adsorbed only on the surface of the needles. Another investigator (Blanchard, 1954, cited by Sutinen *et al*., 1995) found that TCA applied to the foliage of maize was absorbed, but only minor amounts were translocated to other parts of the plant. These data indicate there may be significant differences in uptake and distribution of TCA among vegetable types.

Reimann *et al*. (1996) examined the concentrations of TCA in several vegetables, fruits, and grain samples from Switzerland. The results are presented in Table IV-6. TCA concentrations ranged from < 0.2 µg/kg in fruits and tomatoes to 5.9 µg/kg in spinach.

TCA was only analyzed in wheat flour (0.6 µg/kg) and was below the detection limit of 1.5 µg/kg in breads.

TCA can also be taken up into foodstuffs from the cooking water. In a study conducted under a grant from the US EPA Office of Research and Development (Raymer *et al.*, 2001, 2004), about 3 to 33% of TCA in cooking water was taken up by food during cooking (Table IV-7). In this study, chicken, carrots, green beans, pinto beans and pasta were cooked in water spiked with TCA (50 ppb). Uptake was measured as the difference between the levels of the analyte in food prepared with the spiked water corrected for the levels found in foods prepared with reagent water. When pasta cooked in either untreated or spiked water was rinsed with spiked water, the concentration of TCA was increased. The total uptake for pasta cooked and rinsed in spiked water was about 8% for TCA. TCA is not stable to boiling; the TCA concentration decreased to about 10% of the original value within the first 20 minutes of boiling.

Table IV-6. TCA in Foods

FOOD GROUP	Range of TCA Concentrations µg/kg
Vegetables	< 0.2 (tomato) -5.9 (spinach)
Fruits	< 0.2
Grain	< 1.6 (barley) -4.1 (malt)
Flour/Bread	0.6 (wheat flour) -< 1.5 (bread)

Table IV-7. Uptake of TCA Following Cooking in Spiked Water

Food	TCA (%)
Carrots	24
Green Beans	33
Chicken	14
Pinto Beans	INF
Pasta	5.9

NC = Not Calculated
INF = Interferent - High levels of TCA in the control made estimation of uptake difficult

C.2 Air Intake

TCA is not volatile, and thus, is not likely to be present in indoor air as a result of water use within a home. However, it can be present in outdoor air since it is formed as a combustion byproduct of organic compounds in the presence of chlorine (Juuti and Hoekstra, 1998). Stack gases of municipal waste incinerators have been reported to contain 0.37 - 3.7 µg/m^3 TCA (Mower and Nordin, 1987). In addition, TCA could be a photooxidation product of tetrachloroethylene and 1,1,1-trichloroethane in the atmosphere (Reimann *et al*., 1996; Sidebottom and Franklin, 1996; Juuti and Hoekstra, 1998. However, Sidebottom and Franklin (1996) suggest that atmospheric degradation of chlorinated solvents contributes only a minor amount of TCA to the atmosphere, based on the mechanistic and kinetic evidence, as well as the observed global distribution of TCA in precipitation.

TCA has been detected in rain water at concentration ranges of 0.01 - 1 µg/L (Reimann *et al*., 1996). Sidebottom and Franklin (1996) reported that TCA concentrations in rainwater in remote areas (Antarctic, and the Arctic and sub-Arctic regions) generally ranged from 10 to 100 ng/L (0.01 - 0.1 µg/L). Although there were no data on ambient air concentrations for TCA, an estimate of the concentrations originating in ambient air from could be made from the data on rainwater concentrations. For the purposes of this estimate, the concentration of TCA in rainwater would be considered to be proportional to the concentrations of this chemical in the air since it is very soluble in water (See Chapter II).

C.3 Dermal Exposure

Clemens and Scholer (1992) reported TCA concentrations in 15 indoor and 3 outdoor swimming pools in Germany. The concentrations of TCA were significantly higher in the outdoor pools. The range of TCA concentrations in the water at indoor pools was 3.3 - 9.1 µg/L, with an average of 6.2 µg/L. The range of TCA concentrations in outdoor pools was 46.5 - 100.6 µg/L, with an average of 94.1 µg/L.

Kim and Weisel (1998) measured TCA concentrations in three indoor pools during 1995 and 1996. The highest concentration measured was 871 µg/L, with a mean concentration of 420 µg/L. The TCA concentrations reported by Kim and Weisel (1998) for indoor pools were more than an order of magnitude higher than those reported in the previous Criteria Document (U.S. EPA, 1994) for indoor pools in Germany, and about four times higher than those reported for outdoor pools in Germany. One reason for the difference may be due to the amounts of chlorine used to disinfect swimming pools in Germany compared to the U.S. The differences in concentration may also be the result of differences in the sample collection time relative to chlorination of the water, or addition or exchanges of water in the pools. Under experimental conditions, the formation of TCA increases with reaction time (Pourmoghaddas *et al*., 1993). Therefore, the concentrations may fluctuate depending on the conditions of the pool at the time of sampling. The study by Kim and Weisel (1998) provided evidence for dermal uptake of TCA during exposure to swimming pool water. The absorption data are discussed in further detail in

Chapter III, in the context of the data on absorption and general toxicokinetics following dermal exposure.

D. Overall Exposure

The relative source contribution (RSC) for TCA is derived by application of the Exposure Decision Tree approach published in EPA's *Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health* (U.S. EPA, 2000d). The RSC is the fraction of an individual's total exposure allocated to drinking water. An RSC of 20% accounts for the likelihood of exposure to TCA from sources other than ingested tap water, such as food, ambient air and dermal contact with tap water in the absence of adequate data. The available data are sufficient to demonstrate that food is a relevant exposure source for TCA, in addition to drinking water, however, the data are not adequate to quantify the contributions of each source for an overall assessment of exposure.

To obtain an overall average estimate of TCA concentrations in water, a weighted average for each may be considered, which takes into account the number of plants treating surface water and groundwater (Table IV-1). Therefore, the average TCA concentration can be estimated as 10.38 µg/L (i.e., [304 plants * 13.25 µg/L] + [123 plants * 3.28 µg/L]/[304 + 123 plants]). Using a median intake value of 0.019 L/kg-day based on a median intake of 1.46 L/day and 77 kg body weight (i.e., [1.46 L/day]/77 kg body weight) (U.S. EPA, 1997, 2001), the average intake of TCA through ingestion of drinking water and beverages made with drinking water would be 0.20 µg/kg-day TCA (i.e., 10.38 µg/L * 0.019 L/kg-day).

It is not possible to estimate the concentrations of TCA in the average diet. No data are available from dietary studies similar to the FDA Total Diet Study or duplicate diet studies in which the levels of TCA was analyzed in prepared foods. The available data demonstrate that both compounds are present in some foods and that uptake occurs when foods are cooked or prepared in tap water containing TCA (Raymer *et al.*, 2001, 2004; Reimann *et al.*, 1996). TCA can become incorporated in fruits and vegetables during their growth cycle. TCA is likely to be an indirect additive from the use of hypochlorous acid, sodium hypochlorite and/or calcium hypochlorite with foods during processing and/or preparation either to inhibit microbial growth or as a bleaching agent. The data demonstrate that TCA is likely to be present in the diet, but are insufficient to support an quantitative estimate of exposure by this route.

A recent dermal absorption study of DCA and TCA from chlorinated water suggested that the dermal contribution of the total doses of DCA and TCA from routine household uses of drinking water or household bleach is less than 1% (Kim and Weisel, 1998). Therefore, the contribution to the overall exposure due to dermal absorption from drinking water should be minor. The concentrations of TCA in indoor air are likely to be small because it is nonvolatile and when introduced into outdoor air from combustion or as an oxidation product of chlorinated ethene or ethane solvents will associate with atmospheric moisture (i.e. rain) as a result of its high solubility and low volatility.

The Exposure Decision Tree approach from EPA's *Methodology for Deriving Ambient Water Quality Criteria* (U.S. EPA, 2000d) can be used for determining the RSC for TCA. There are enough information to identify drinking water and diet as the dominant exposure pathways for TCA. However, there are not enough data available to characterize exposure from the dietary route. Ambient air and dermal contact with water in showering or bathing as possible minor exposure routes that also lack sufficient data for quantification. Accordingly, in accordance with the Exposure Decision Tree approach (U.S. EPA, 2000d), the default RSC factor of 20% is used for TCA, indicating that drinking water exposure is assumed to account for 20% of total exposure.

D.1 Body Burden

The body burden from TCA is complicated by the endogenous production of TCA from several common environmental contaminants that produce TCA during metabolism (Bruning *et al*., 1998; Skender *et al*., 1994; Hajimiragha *et al*., 1986; Vartiainen *et al*., 1993; Ziglio, 1981; Ziglio *et al*., 1983; Humbert *et al*., 1994). Trichloroethylene (TCE), tetrachloroethylene (PERC), 1,1,1-trichloroethane, 1,1,1-trichloroethanol and chloral hydrate all produce TCA endogenously as a result of metabolism. TCE and PERC are common contaminants in ambient air and drinking water (US EPA, 2003) and thus could increase the TCA body burden. Ambient air concentrations of TCE ranges from 0.01 to 3.9 $\mu g/m^3$ based on monitoring from 25 states (US EPA, 1999a) and levels of PERC were 16 ppb in rural and remote areas, 0.79 ppb in urban and suburban areas and 1.3 ppb in areas near emission sources (US EPA, 1999b). Ambient air exposure levels were not available for 1,1,1-trichloroethane. The chlorinated solvents are also common drinking water contaminants. Analysis of monitoring data on chlorinated ethane solvents contaminants as part of the EPA six-year review found that TCE was identified at least once during potable water quarterly monitoring for 2.61% of the systems evaluated, PERC was identified by 3.36% of the systems and 1,1,1-trichloroethane for 2.50% of systems (US EPA, 2003). Additional endogenous exposure would result when chloral hydrate and or 1,1,1-trichloroethanol were present and via disinfection byproducts in tap water disinfected with chlorine.

TCA does not appear to accumulate in tissue, but its slow excretion rate (see Chapter III) means that some accumulation could occur if exposure to TCA, or to compounds metabolized to TCA, is higher than the TCA excretion rate. The nature of the binding of TCA to proteins has not been established. To the extent that the binding is electrostatic, the release of bound TCA from protein binding is possible during the turnover of cellular proteins. Rapid clearance was observed following low-dose dermal exposures (Kim and Weisel, 1998), suggesting limited potential for bioaccumulation at doses likely to result via the dermal exposure pathway).

E. Summary

Data from the ICR database (U.S. EPA, 2000a) indicate that concentrations of TCA were consistently lower in groundwater than in surface water. The mean concentrations of TCA were

3.28 and 13.25 µg/L in groundwater and surface water, respectively. The mean concentrations of MCA were 0.76 and 1.28 µg/L in groundwater and surface water, respectively.

The data for surface water plants indicate that ozonation, followed by chlorine or chloramine in the distribution system, could decrease mean TCA concentrations more than using free chlorine alone. TCA concentrations in groundwater did not differ significantly among the non-ozonation purification methods. There were no significant differences between the two treatments using ozonation in treating surface water for TCA. A regression analysis of the ICR data indicates that there is no significant correlation ($\alpha = 0.05$) between influent TOC concentration and the mean concentration of TCA in surface or groundwater. ICR data also indicate that mean seasonal concentrations of TCA in groundwater were not significantly different, nor were there any consistently significant differences between the mean seasonal concentrations of these chemicals in surface water.

In addition to TCA concentrations in drinking water, there are some data on TCA concentrations in air, food, and swimming pool water. TCA concentrations average 13.25 and 3.28 µg/L in surface water and groundwater, respectively. Very limited data are available on the concentrations of TCA in foods, but they demonstrate that the concentrations in foods may contribute to the overall dose. Inadequate data are available regarding concentrations of TCA in ambient air. Although dietary exposures are potentially significant sources of exposure to TCA, there is a lack of sufficient monitoring data to quantify these exposures. Therefore, EPA is using a default 20% RSC for tap water in accordance with the Exposure Decision Tree approach.

Very limited data were available on the levels of TCA in the blood or urine resulting from direct exposure to TCA. Some data that show that chlorinated solvents contribute to the total TCA body burden, these data were not appropriate for estimation of TCA human body burden because neither environmental intake nor the kinetics of absorption and distribution of TCA produced endogenously were taken into account when the source of TCA was a metabolite from a chlorinated solvent. Exposure to chloral hydrate and trichloroethanol as disinfection byproducts can also add to the TCA body burden since they are metabolized to TCA.

Chapter V. Health Effects in Animals

A. Short-Term Exposure

A.1 Oral

In short-term toxicity studies for TCA (Davis, 1986; Davis, 1990), high doses resulted in decreased food consumption and body weight loss. The liver was frequently identified as a target organ for TCA toxicity, with peroxisome proliferation being the primary endpoint evaluated (Goldsworthy and Popp, 1987; DeAngelo *et al*., 1989; Sanchez and Bull, 1990). Alterations in intermediary carbohydrate metabolism (e.g., decreased lactate levels in several tissues) were also observed (Davis, 1990).

Miyagawa *et al*. (1995) conducted acute toxicity testing for dose-range finding as part of a study on TCA-induced replicative DNA synthesis. Groups of male B6C3F1 mice (4 or 5/dose) were administered a single oral-gavage dose of TCA to determine the maximally tolerated dose (MTD) which was set at about half the $LD_{50.}$ The MTD for TCA was estimated to be 500 mg/kg.

Recent studies have also evaluated the effects of TCA on the liver. In an acute study by Austin *et al*. (1996), male B6C3F1 mice (6/group) were treated with a single oral dose of TCA (0, 30, 100, or 300 mg/kg). Mice were deprived of food for 3 hours prior to dosing. Liver nuclear DNA was extracted to assess increases in 8-hydroxydeoxyguanosine (8-OHdG) adducts, a measure of oxidative damage to DNA resulting from oxidative stress. TCA has been shown to induce lipid peroxidation in rodents (Larson and Bull, 1992) and compounds that produce oxidative stress also increase 8-OHdG, which is capable of inducing DNA base transversions that might be involved in the carcinogenic process (Chang *et al*., 1991). A significant increase in 8-OHdG in nuclear DNA in the liver was observed in the 300-mg/kg group at 8-10 hours post-dosing. The maximum 8-OHdG level was observed at 8 hours, and was an increase of approximately one-third (estimated from Figure 3 in the paper) over controls. The 8-OHdG levels in groups dosed with 30 or 100 mg/kg were not reported.

The study authors contrasted the profile of oxidative-DNA damage induced by TCA in this study with TCA-induced levels of thiobarbituric acid-reactive substances (TBARS, an indicator of lipid peroxidation) reported in a previous study (Larson and Bull, 1992). In the earlier study, Larson and Bull (1992) reported a maximum concentration of TBARS 9 hours post-dosing in the livers of mice given 2000 mg/kg TCA. The Larson and Bull (1992) study also reported that a single oral dose of TCA induced TBARS levels by 1.15-, 1.7-, 2-, and 2.7-fold over controls at doses of 100, 300, 1000, and 2000 mg/kg, respectively. Austin *et al*. (1996) suggested that the ability of haloacetates to increase both TBARS and 8-OHdG levels indicates that oxidative stress may be related to their hepatocarcinogenicity. The concordance between TBARS and 8-OHdG levels also suggested a common mechanism of induction of these two markers. Neither a No-Observed-Adverse-Effects Level (NOAEL) nor a Lowest-Observed-

Adverse-Effects Level (LOAEL) were identified for this study because no standard measures of liver toxicity (or other toxicity endpoints) were conducted.

Parrish *et al*. (1996) evaluated the ability of haloacetic acids to induce oxidative DNA damage in the livers of mice. Male B6C3F1 mice (6/group) were exposed to 0, 100, 500, or 2000 mg/L TCA in drinking water for either 3 or 10 weeks. The study authors did not estimate the doses resulting from exposure to treated drinking water. However, based on default water-intake values of 0.25 L/kg/day for male B6C3F1 mice (U.S. EPA, 1988), the corresponding doses were 0, 25, 125, and 500 mg/kg/day. Body weight and liver weight were evaluated and several indicators for peroxisome proliferation were measured, including cyanide-insensitive palmitoyl-CoA oxidase activity and increased 12-hydroxylation of lauric acid, which have been identified in other studies as "classical" responses to peroxisome proliferators (Parrish *et al.* 1996). Spectrophotometric and differential centrifugation methods were used to assess these endpoints. The level of 8-OHdG in liver nuclear DNA was also evaluated as an indicator of oxidative DNA damage.

No differences in body weight were observed for any of the treatments. The absolute liver weight was increased at the high dose, and relative liver weight was increased at the mid and high dose (by 13% and 33%, respectively) following exposure for 3 weeks ($p<0.05$). After 10 weeks of exposure, absolute and relative liver weights were significantly increased at the mid dose and higher, (increases of 12% and 35%, respectively, for relative liver weights). No histopathological examination or clinical chemistry were performed. Significant dose-related increments in cyanide-insensitive palmitoyl-CoA oxidase activity were observed in mice treated with all TCA doses for 3 weeks; these increases persisted when treatment was extended to 10 weeks. Significantly increased 12-hydroxylation of lauric acid was also observed after both 3 and 10 weeks of TCA exposure (statistically significant at the high dose), whereas 8-OHdG levels were unchanged at both time periods. Thus, oxidative damage to genomic DNA as measured by 8-OHdG adducts did not occur with prolonged TCA treatment even though peroxisome proliferation was induced, as indicated by increased palmitoyl Co-A oxidase activity and 12-hydroxylation of lauric acid.

The authors concluded that the lack of an increase in 8-OHdG indicated that this type of DNA base damage was not likely to be associated with the initiation of cancer by TCA; either the formation of these adducts was inhibited or their repair was enhanced with continued TCA treatment. The increased relative liver weight of approximately 10% at the mid dose (125 mg/kg/day) was accompanied by a significant increase in palmitoyl CoA oxidase activity, but not 12-hydroxylation of lauric acid. The severity of these changes at the high dose was much greater, with relative liver weight increasing roughly 35% over controls, and significant increases in both indicators of peroxisome proliferation. Liver histopathology was not conducted in these experiments. However, based on significant increases in relative liver weight accompanied by markers of peroxisome proliferation, the mid-dose of 125 mg/kg/day is considered a LOAEL. The low-dose of 25 mg/kg/day is considered a NOAEL.

Austin *et al*. (1995) tested whether TCA pretreatment would alter the lipid-peroxidation response of a subsequent acute dose of TCA. They also explored the relationship between TCA-induced lipid peroxidation and the ability of TCA to induce markers of peroxisome proliferation or cytochrome P450s following short-term treatments. Male B6C3F1 mice (n=6/group) were treated with 0 or 1000 mg/L TCA for 14 days, approximately 0 or 250 mg/kg/day, based on the default water intake of 0.25 L/kg/day for male B6C3F1 mice (U.S. EPA, 1988). For the lipid-peroxidation experiments, the pretreated mice were administered 300 mg/kg of TCA, or an equivalent volume of distilled water by gavage (control) as an acute challenge. Animals were sacrificed 9 hours after the acute challenge. The following endpoints were evaluated for the animals given treatments for 14 days: (1) lipoperoxidative response in mouse-liver homogenate, as measured by the production of TBARS; (2) indicators of peroxisome proliferation, as measured by increased palmitoyl-CoA oxidase (PCO) activity, increased catalase (CAT) activity; and changes in 12-hydroxylation of lauric acid (an indicator for the activity of cytochrome P450 4A (CYP4A); and (3) activity of CYP2E1 and protein levels for a panel of cytochrome P450s, as described in Section III.C. (Toxicokinetics - Metabolism). In addition to measurements following 14 days of treatment, TBARS levels were also measured for the acute-challenge experiments.

No changes in water consumption or body weight were observed, although relative liver weight was increased by 29% after 14 days of TCA treatment. TCA-treated mice had a lower mean TBARS level as compared with controls, but the difference was not statistically significant. In the acute challenge experiment, TCA-pretreated mice exhibited a significant decrement in TBARS in liver homogenates following acute dosing with TCA compared with animals that received the same acute challenge, but which had not been pretreated. In contrast to the decrease in TBARS induced by TCA pretreatment, PCO, CAT, and CYP4A activities were significantly increased by pretreatment with TCA. These data demonstrate that treatment of mice with TCA reduced lipoperoxidative responses but increased other markers that have been associated with peroxisome proliferation. The authors suggested that reduction in the TBARS response observed in TCA-pretreated animals resulted from activities associated with peroxisome proliferation and might be related to a shift in the expression of P450 isoforms, such as CYP4A. Peroxisomes were not measured directly, however. Based on significant increases in relative liver weight and several indirect markers of peroxisome proliferation (PCO, CAT, and CYP4A activities), the single dose tested of 250 mg/kg/day is considered a LOAEL for this study.

In summary, the ability of TCA to induce oxidative-stress responses such as lipid peroxidation and oxidative DNA damage, and the relationship between these responses and indicators of peroxisome proliferation or altered cytochrome P450 activities has been tested in a series of studies following acute or short-term TCA dosing in mice (Larson and Bull, 1992; Austin *et al*., 1995; Austin *et al*., 1996; Parrish *et al*., 1996). TCA induces both lipid peroxidation (TBARS) and oxidative DNA damage (8-OHdG) following administration of single oral doses. However, these increases appear transient, since neither lipid peroxidation (Austin *et al*., 1995) nor 8-OHdG formation (Parrish *et al*., 1996) were increased in multiple-dose studies.

In contrast, responses associated with peroxisome proliferation are induced following TCA dosing for up to 10 weeks (Austin *et al*., 1995; Parrish *et al*., 1996). These results suggest that peroxisome proliferation, but not oxidative-stress responses, may be associated with liver toxicity observed in short-term studies.

Dees and Travis (1994) evaluated the ability of TCA to induce DNA synthesis in the livers of male and female B6C3F1 mice. Mice (5/sex/dose) were given 11 daily gavage doses of 0, 100, 250, 500, or 1000 mg/kg/day TCA in corn oil. Twenty-four hours after the last dose, [^{3}H]thymidine was administered intraperitoneally (i.p.). Six hours later, the mice were sacrificed and their livers removed. There were no clinical signs of toxicity at the time of sacrifice, and no significant effects on body weight or body-weight gain. Absolute and relative liver weights were significantly increased in all of the tested groups when compared to controls, but no dose-response was apparent. In males, the relative liver weight was increased by 15% (at 500 mg/kg/day) to 28% (at 250 mg/kg/day), and the increases were not dose-related. The relative liver weight in females was increased by 9% or less at all doses.

Histopathological changes were observed for both males and females only at 1000 mg/kg/day. Histopathological changes included a slight increase in the eosinophilic cytoplasmic staining of hepatocytes near the central veins. The increase in eosinophilic staining was accompanied by a loss of cytoplasmic vacuoles. In the intermediate zone, subtle changes in cellular architecture were noted, including disarray of the parallel pattern of hepatic cords. The authors suggested that this was indicative of areas of nodular cellular proliferation. In TCA-treated mice, [^{3}H]thymidine incorporation (observed autoradiographically) was mostly localized in the intermediate zone in cells that resembled mature hepatocytes, while labeling in controls occurred primarily in the peri-sinusoidal cells. Similar patterns of labeling were observed in male and female mice. In addition, mitotic figures (indicative of dividing cells) were observed in the livers of TCA-treated mice, but not in controls, and these dividing cells had often incorporated the radiolabel into the DNA; these effects indicate the labeling of newly replicated DNA synthesis, rather than labeling of damaged DNA. The number of mature hepatocytes labeled with [^{3}H]thymidine appeared to increase with increasing TCA dose, reaching a maximum of approximately 2.5-fold increase at 1000 mg/kg/day (no statistical analysis was reported).

Incorporation of [^{3}H]thymidine in extracted liver DNA also increased as TCA dose increased, with the effect significant in males at all doses and in females at $\geq$250 mg/kg. No difference in total liver DNA content (mg DNA/g liver) was observed. Peroxisomes were not measured. The authors concluded that their results are consistent with an increase in DNA synthesis and cell division/proliferation in response to TCA treatment. The authors further suggested that because only slight histopathological effects were observed at the highest dose, it was unlikely that the increased DNA synthesis and cell division were secondary to tissue repair. Based on the increased relative liver weight and DNA synthesis in male mice supported by the histopathological evidence of cell proliferation, 100 mg/kg/day is considered to be a minimal LOAEL for this study. This dose was judged a minimal LOAEL because the observed effects were of mild severity, the increase in DNA labeling was fairly small (although statistically

significant), and clearly adverse effects such as liver histopathological changes were observed only at the highest dose tested (1000 mg/kg/day).

Acharya *et al*. (1995) evaluated liver and kidney toxicity of TCA as part of a study on the interactive toxicity of tertiary butyl alcohol and TCA. Young male Wistar rats (5-6/dose) were exposed to water containing 0 or 25 ppm (3.8 mg/kg/day), assuming a default water intake of 0.15 L/kg/day (U.S. EPA, 1988) TCA for 10 weeks. In the TCA-treated animals, terminal body weight was decreased (to approximately 83% of controls) in the absence of changes in food consumption (data not shown). Little, if any, TCA-induced liver cytotoxicity was observed. Relative liver weight was not significantly different in TCA-treated animals. No significant changes were detected in serum aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, or acid phosphatase activities. In contrast to the serum markers of liver necrosis, serum indicators of lipid and carbohydrate homeostasis were affected by TCA. Succinate dehydrogenase activity was increased by roughly 30%. Serum total triglyceride and serum glucose levels were also significantly increased, without any change in serum cholesterol levels. Liver triglyceride and cholesterol levels were significantly decreased, while liver-glycogen levels were dramatically increased (roughly 8-fold). The enzymatic basis for increased hepatic glycogen accumulation remains unclear.

There was little evidence for induction of oxidative stress in the liver. Kidney, but not liver, glutathione levels were decreased to approximately 66% of control values and no increase in lipid peroxidation was observed in the liver. In a follow-up study using the same exposure protocol (Acharya *et al*., 1997), histopathological changes in the liver and kidney were evaluated. The authors noted that only marginal hepatic alterations were observed due to the fact that sub-toxic doses were administered to the TCA-only treatment group. Liver histopathological changes that were noted included centrilobular necrosis, hepatocyte vacuolation, loss of hepatic architecture, and hypertrophy of periportal region. Hypertrophy of the periportal region may have accounted for the observed increase in liver weight. The magnitude of these changes was limited, consistent with the absence of effects on serum-liver enzymes in the earlier study. Neither glycogen accumulation nor peroxisomes were assessed in this histopathological study.

Histopathological changes were also noted in the kidneys of TCA-treated animals, and included degeneration of renal tubules with syncitial arrangement of the nucleus in the epithelial cells, degeneration of the basement membrane of Bowman's capsule, diffused glomeruli, vacuolation of glomeruli, and renal tubular proliferation in certain areas. Based on the liver and kidney histopathological changes at the single dose tested, the study authors indicated that TCA is a liver and kidney toxicant.

Taken together, the two studies by Acharya *et al*. (1995; 1997) suggest that the single dose tested, 3.8 mg/kg/day, is an apparent LOAEL. However, a number of questions preclude a definitive determination of the LOAEL. First, the authors noted a lack of increase in liver enzyme activity in the earlier study. Although liver histopathological changes were observed,

they were described as "only marginal" by the authors. The authors did not discuss the severity of the histopathological changes in relationship to untreated controls, and no incidence data were provided. Therefore, it is not clear whether the effects observed were adverse. Due to this uncertainty, 3.8 mg/kg/day can be best described an equivocal LOAEL.

Laughter and coworkers (2004) expanded the investigation of the role of PPAR α in the spectrum of responses associated with mouse liver cancer following TCE, TCA, and DCA exposure. Male mice, either SV129 wild-type or PPAR α-null strain (lacking the receptor), 9weeks in age, were used in three separate studies. However, only one of these studies examined the direct response to TCA. In that study the mice were given TCA at 0.25, 0.5, 1.0, or 2.0 g/L in drinking water for 7 days. At the end of the exposure period, the mice were sacrificed, liver and body weights measured, and liver slices fixed for histopathology. Liver to body weights were not increased significantly in wild-type mice given TCA in drinking water, or in the PPAR α-null mice. Centrilobular hepatocyte hypertrophy was found in the wild-type but not in the PPAR α-null mice in the highest dose group (2 g/L). Induction of lipid metabolism enzymes, such as Cyp4a and acyl-CoA oxidase (ACO) occurred in the livers of wild-type mice but not the null mice. These data indicate that effects in mouse liver following administration of TCA were dependent on the peroxisomal proliferative-inducing qualities of these compounds and that PPAR α was necessary for those effects.

The role of exposure to TCA in the development of autoimmune diseases was studied by Blossom *et al.* (2004). The authors used a mouse model with autoimmune-prone MRL +/+ which develop lupus late in life (50% mortality in 17 months) and the MRL lpr/lpr mouse strain, which develops the disease early in life (50% mortality in 6 months). Groups of 6-week old mice from either strain were administered TCA diluted in water for 4 weeks at the following concentrations: 0, 0.1, or 0.9 mg/mL (0, 27 and 205 mg/kg/day doses, calculated by the authors). A phenotypic analysis of splenic and lymph node cells from the MRL +/+ mice indicated that TCA treatment increased the number of CD4+ cells that expressed the $CD62L^{lo}$ marker, an adhesion molecule whose loss of expression on T lymphocytes indicates an activated or effector T cell. However, there was no dose-response seen in TCA treatment, as both concentrations (0.1 and 0.9 mg/mL) caused the same increase (55%) in cells expressing the marker. Compared to cells from control mice, these cells also showed increased production of the cytokine IL-2, but not IL-4, indicating that the CD4+ cells have been skewed towards a TH1-like immune response and also had an increased production of interferon-γ, a pro-inflammatory cytokine. Both of these changes are hallmarks of inflammatory autoimmune diseases (Janeway *et al.*, 1999). The activation state of B cells was unaffected, as no increase in B cell expression of MHC (major histocompatibility class) II antigens was noted. More importantly, TCA exposure resulted in decreased apoptosis (~68% of the CD4+ T cells from MRL +/+ mice compared to 84.5% from non-treated mice). As noted by the study authors, the data indicate that short-term exposure to low levels of TCA in drinking water were adequate to promote CD4+ T-cell activation and to prevent activation-induced cell death, thereby magnifying the TH1 skewing of the immune response.

A.2 Dermal

TCA is a skin irritant. The ability of a variety of carboxylic acids to cause skin corrosion was investigated using multivariate quantitative structure-activity relationship (QSAR) analysis (Eriksson *et al*., 1994). Fifteen chemicals, including TCA, were tested for cutaneous corrosion on adult rabbits following a 1-hour exposure to TCA (applied to bare shaved skin and occluded by a glass filter to prevent evaporation). The lowest-observed-effect concentration (LOEC) for TCA-induced corrosion was identified as 1.5 M.

A.3 Inhalation

No short-term toxicity studies for TCA were identified for exposure by the inhalation route.

B. Long-Term Exposure

B.1 Oral

Long-term oral toxicity studies for TCA have primarily identified effects on the liver, including increased liver weight, peroxisome proliferation, and a variety of histopathological lesions (Mather *et al*., 1990; Bull *et al*., 1990; Bhat *et al*., 1991).

DeAngelo *et al*. (1997) reported on the noncancer effects of TCA in a study on the tumorigenicity of TCA in male F344 rats. Groups of 50 rats were administered TCA in drinking water (adjusted to physiologic pH), at 0, 50, 500, or 5000 mg/L, resulting in time-weighted mean daily doses of 0, 3.6, 32.5, or 364 mg/kg for 104 weeks, beginning at 28-30 days of age. Interim sacrifices were conducted at 15, 30, 45, and 60 weeks, and gross lesions in the body and internal organs were examined; the survivors were sacrificed at 104 weeks. There were no significant differences in water consumption or animal survival between the control and treatment groups. Exposure to the high dose of TCA resulted in a significant decrease in body weight of 11% at the end of the study. The absolute, but not relative, liver weight was decreased at the high dose, probably reflecting the decrease in body weight. Absolute and relative weights of the kidney, spleen, or testes were unchanged. Mild hepatic cytoplasmic vacuolization was noted in the two low-dose groups, but not in the high dose group. The severity of hepatic necrosis was increased mildly in the high-dose animals. No treatment-related histopathological changes were noted for the kidney, spleen, or testes. Aspartate aminotransferase (AST) was significantly decreased at the mid dose and alanine aminotransferase (ALT) was significantly increased at the high dose at study end. Because increased serum ALT or AST levels reflect hepatocellular damage, the increased ALT at the high dose is considered an adverse effect, while a non-dose related decrease of AST is not. Peroxisome proliferation in the livers of animals exposed to the high dose of TCA was increased significantly, based on a 2-fold increase in cyanide-insensitive palmitoyl CoA oxidase activity. There was no evidence of any exposure-related increase in hepatocyte proliferation, based on [^{3}H]thymidine incorporation data. Based on the significant

decrease in body weight (≥10%), minimal histopathology changes, increased serum ALT levels, and increased peroxisome proliferation, the high dose of 364 mg/kg/day is considered the LOAEL, and the mid dose of 32.5 mg/kg/day is considered the NOAEL.

Pereira (1996) administered 0, 2.0, 6.67, or 20.0 mmol/L TCA (0, 327, 1090, or 3268 mg/L) (neutralized to pH 6.5-7.5) in drinking water to female B6C3F1 mice from 7-8 weeks of age until sacrifice after 360 days (51 weeks) or 576 days (82 weeks) of exposure. The estimated doses resulting from exposure to treated drinking water were not presented. However, based on the default water intake for female B6C3F1 mice of 0.24 L/kg/day (U.S. EPA, 1988), the doses can be estimated as 0, 78, 262, and 784 mg/kg/day. The study was designed to assess the hepatocarcinogenicity of TCA and the only non-cancer toxicological endpoints measured were body weight and liver weight. Drinking-water consumption was decreased only for the first week for the high-dose group. Body weight was decreased beginning after 51 weeks of treatment with 20 mmol/L (784 mg/kg/day) TCA. Estimating from data presented in Figure 1 of the study, body weights were decreased by approximately 10% on sporadic occasions beginning at week 51, and were statistically significant ($p<0.05$) at various time points over this period. The decrease in body weight at study termination was approximately 10%. Relative liver weight increased with dose (linear regression coefficient, $r = 0.991$). The relative liver weights of the high-dose group increased by roughly 40% over controls at 360 days, and liver weights for the mid- and high-dose groups increased by roughly 25% and 60% over controls, respectively, after 576 days. Based on the increase in liver weight of approximately 25%, the NOAEL for this study is 78 mg/kg/day, and the LOAEL is 262 mg/kg/day. The adversity of the liver weight increase at 262 mg/kg/day is supported by short-term studies in B6C3F1 mice that have reported glycogen accumulation (Sanchez and Bull, 1990), increased hepatocyte labeling (Dees and Travis, 1994), and peroxisome proliferation (Parrish and Bull, 1996) at TCA doses that increased liver weights.

B.2 Dermal

No long-term toxicity studies for TCA were identified for exposure by the dermal route.

B.3 Inhalation

No long-term toxicity studies for TCA were identified for exposure by the inhalation route.

C. Reproductive/Developmental Effects

No studies were identified on the reproductive toxicity of TCA administered via the oral, inhalation, or dermal routes. In a developmental toxicity study with pregnant Long-Evans rats (20-21/dose) exposed via gavage to doses of 0, 330, 800, 1,200 or 1,800 mg/kg/day on gestation

days (gd) 6-15 (PBPK *et al*., 1989), TCA induced both maternal toxicity (decreased body weight and increased spleen and kidney weight) and developmental toxicity (decreased fetal weight and length, increased cardiovascular malformations, particularly levocardia and interventricular septal defects, and increased total soft-tissue malformations) at 330 mg/kg/day (LOAEL) and above. No NOAEL could be determined as this dose level was the lowest dose tested.

In a report investigating the cardiac teratogenicity of trichloroethylene metabolites, Johnson *et al*. (1998) exposed pregnant Sprague Dawley SD rats to 0 (n=55) or 2730 (n=11) mg/L TCA in neutralized drinking water on gd 1-22. The authors estimated the doses to be 0 or 291 mg/kg/day, based on the average daily amount of water consumed by the animals. Maternal toxicity was evaluated by clinical observation and maternal weight gain. Dams were sacrificed on gd 22 and implantation sites, resorption sites, fetal placements, fetal weights, placental weights, fetal crown-rump lengths, gross fetal abnormalities and abnormal fetal abdominal organs were recorded. In addition, the fetal hearts were removed, dissected, and examined microscopically for abnormalities. No signs of maternal toxicity were reported. Although the authors reported that the weight gain during pregnancy of treated females was not significantly different from controls, the average maternal weight gain for TCA-exposed animals was 84.6 g as compared with 122 g for control animals, representing a 30% decrease in maternal body weight gain. Thus, it is not clear why this reduction was not reported as statistically significant. Nonetheless, a decrease of this magnitude in body weight gain during pregnancy is considered to be toxicologically significant. Average daily drinking water consumption was reported as 38 mL/day in treated rats as compared with 46 mL/day in control rats; this difference was not reported as statistically significant, however, it was unclear whether water intake was evaluated statistically.

Statistically significant increases were reported in average resorption sites (2.7 resorptions/litter in treated animals, compared to 0.7 in the controls), total number of resorptions (30 resorptions reported among 11 treated females as compared with 40 resorptions among 55 control females [an average of 2.7/litter in TCA-treated dams vs. 0.73 in control]), and average implantation sites (defined as sites where the fetus was implanted but did not mature) of 1.1 implantation sites/litter, compared to 0.2 in the controls. TCA-treated dams had a mean of 10.5 fetuses/litter compared to control dams with 11.3 fetuses/litter; the difference was not reported as statistically significant. The number of maternal rats with abnormal fetuses was 7 out of 11 (0.64/litter) for TCA-treated animals as compared with 9 out of 55 (0.16/litter) for controls. No significant differences were reported in the numbers of live or dead fetuses, fetal weight, placental weight, fetal crown-rump length, fetal external morphology, or fetal gross external or noncardiac internal congenital abnormalities; however, quantitative data for these endpoints were not reported in the paper.

Cardiac abnormalities were evident in 10.5% of the fetuses in the TCA group, compared to 2.15% of the controls. As determined by the authors, the incidence of cardiac malformations was significantly greater in treated as compared to control rats on both a per-fetus basis ($p = 0.0001$) and a per-litter basis ($p = 0.0004$). Complete fetal examinations for internal or skeletal

abnormalities were not conducted. The study is limited by the use of one-dosed group with only11 dams exposed to TCA. Based on the toxicologically significant decrease in maternal body weight, 291 mg/kg/day is considered to be a maternal LOAEL. Based on an increase in cardiac malformations, the developmental LOAEL, occurring at a maternally toxic dose, is 291 mg/kg/day.

Fisher *et al.* (2001) also reported on the results of a single dose developmental study in Sprague-Dawley rats. Doses of 0 or 300 mg/kg-day were given by oral gavage on gd 6-15 to groups of 19 pregnant animals. Dams were sacrificed on gd 21; body weight, uterine weight, number and viability of fetuses, and number of implantation and resorption sites were recorded. Full term fetuses were removed, and sex, fetal weight (per fetus and per litter), and number of fetuses per dam were recorded. The heart of each full-term fetus was examined for cardiac malformations using a detailed cardiac micro-dissection technique. The single dose evaluated produced maternal toxicity as indicated by decreased body weight gain of the dams ($p \leq 0.05$, approximately 17% relative to controls). The number of implantations, percent of dams with an early resorption, and number of fetuses per litter were similar to control values. Mean fetal body weight was significantly less than controls ($p \leq 0.05$, approximately 8%). Unlike the study by Johnson *et al.* (1998), the heart malformation incidence in the TCA-treated group was similar to controls; 3.3% (9/269) of the fetuses and 42% (8/19) of the litters from TCA-treated animals were affected compared to 2.9% (8/273) of fetuses and 37% (7/19) of litters from control animals. These data identify a maternal LOAEL of 300 mg/kg-day based on significantly reduced body weight gain and a developmental LOAEL of 300 mg/kg-day based on statistically significantly reduced mean fetal body weight on a per litter and per fetus basis.

No studies on the developmental toxicity of TCA were identified for exposure by the dermal or inhalation routes.

In support of the animal data, TCA has also been tested in a number of alternative screening models for assessing potential developmental toxicity. Hunter *et al*. (1996) conducted a 24-hour exposure of 3-6 somite staged CD-1 mice embryos to TCA at concentrations of 0, 0.5, 1, 2, 3, 4, or 5 mM. Effects on neural-tube development were observed at concentrations lower than effects on other morphological processes. Other statistically significant dysmorphology included eye defects, pharyngeal-arch defects, and heart defects. TCA produced abnormal embryonic development at concentrations greater than or equal to 2 mM, with a very steep dose-response slope from 2 to 5 mM. No adverse effects were observed at 1 mM or below, and defects of the eyes, arches, and heart were seen only in embryos that also had very high rates of neural tube defects. The observed effects were not due to low pH, since they were not seen when HCl was added to bring the culture medium to similar pHs.

The potential developmental toxicity of TCA was studied *in vitro* using a rat whole-embryo culture system by Saillenfait *et al*. (1995). Groups of 10 to 20 explanted embryos from Sprague-Dawley rats on gestational day 10 were cultured for 46 hours in 0, 0.5, 1, 2.5, 3.5, 5, or 6 mM TCA. TCA induced statistically significant, concentration-related decreases in the growth

and development parameters of the cultured embryos. Yolk sac diameter was significantly decreased, beginning at a concentration of 1 mM. Other developmental measures, including crown-rump length, head length, somite (embryonic segment) number, protein content, and DNA content, were significantly decreased beginning at 2.5 mM and above. The total number of malformed embryos was increased beginning at 2.5 mM. At 2.5 mM, 55% of the embryos had brain defects, 50% had eye defects, 32% had reduced embryonic axis, 55% had reductions in the first branchial arch, and 36% had otic (auditory) system defects.

TCA has also been evaluated in developmental toxicity screening assays in non-mammalian systems. TCA was evaluated using the FETAX assay in a study that assessed the developmental toxicity of trichloroethylene and its metabolites (Fort *et al*., 1993). Early *Xenopus laevis* embryos were exposed to a range of TCA concentrations for 96 hours. The LC_{50} was 4060 mg/L and the EC_{50} for malformations was 1740 mg/L. Malformations were observed at concentrations greater than 1500 mg/L, and included gut miscoiling, craniofacial defects, microophthalmia, microencephaly, and various types of edema.

Fu *et al*. (1990) studied the developmental toxicity potential of TCA and MCA using a regeneration assay from reaggregated *Hydra* cells. The *Hydra* system is an *in vitro* assay that determines the degree to which a test chemical can perturb embryonic development at maternally subtoxic doses and thus is considered to be useful as a prescreening assay for developmental toxicity (Fu *et al*., 1990). In this study, both intact adult *Hydra* and artificial "embryos" (pellets of the disassociated and randomly reaggregated, terminally differentiated and pluripotent stem cells of *Hydra attenuata*) were treated with TCA at concentrations ranging from 10^{-3} to 10^{3} mg/L. The minimal effective toxic concentration for adults (A) and artificial embryos (D) were determined, and the A/D ratio was evaluated as a developmental-toxicity hazard index. The TCA treatment resulted in an A/D ratio of 1.0. This result suggested that the developing *Hydra* are no more sensitive to TCA than adult *Hydra*, and indicates that in this test system TCA does not selectively interfere with embryonic development at adult subtoxic doses. According to the authors (Fu *et al*., 1990), the *Hydra* system is designed to overestimate developmental hazard potential and is considered to be more sensitive to developmental toxicity than most *in vitro* mammalian test systems; its primary utility is to identify compounds for *in vivo* developmental toxicity testing. Based on these results, TCA would not be considered a high-priority compound for further testing *in vivo*.

One *in vitro* study was identified that suggested that TCA might decrease fertilization. The effect of TCA on *in vitro* fertilization was examined in hybrid C57BL6 x DBA/2 (B6D2F1) mice (Cosby and Dukelow, 1992). TCA was constituted in culture medium to yield concentrations of 100, 250, or 1000 ppm on a v/v basis (approximately 160, 400, or 1600 mg/L) and incubated with mouse oocytes and sperm for 24 hours. Each culture dish was subsequently scored for percentage oocytes fertilized. The percent of oocytes fertilized was significantly decreased compared to controls at 250 mg/L ($p<0.025$) and at 1000 mg/L ($p<0.001$).

D. Genotoxicity

Negative results were reported for TCA in the Ames assay in strain TA100 in the absence of metabolic activation (Rapson *et al*., 1980); a more complete testing in this system was not conducted. In a more extensive analysis, TCA was found to be non-mutagenic in TA98, TA100, and RSJ100 strains, in the absence or presence of metabolic activation at concentrations ranging between 0.1-100 mM (Kargalioglu *et al.*, 2002). In contrast, TCA was positive for the induction of bone-marrow micronuclei in mice (Bhunya and Behera, 1987), and induced a weak increase in "SOS DNA repair" (an inducible error-prone repair system) in *Salmonella typhimurium* strain TA1535 in the presence of rat liver S9 (Ono *et al*., 1991). Earlier studies on the ability of TCA to induce single-strand breaks have produced mixed results (Nelson and Bull, 1988; Chang *et al*., 1991).

A subsequent evaluation of the genotoxicity of haloacetic acids formed during drinking-water chlorination and/or ozonation was conducted by Giller *et al*. (1997) using three short-term assays: the SOS chromotest (which measures DNA damage and induction of the SOS repair system) in Escherichia coli PQ37, with and without metabolic activation (S9); the Ames fluctuation test in *S. typhimurium* TA100, with and without metabolic activation; and the newt (*Pleurodeles waltl* larvae) micronucleus test. In the SOS chromotest, conducted at concentrations of TCA ranging from 10 to 10,000 µg/mL, TCA did not show any genotoxic effect with or without metabolic activation. In the Ames fluctuation test, TCA demonstrated mutagenic activity in the absence of S9 at noncytotoxic concentrations ranging from 1750 to 2250 µg/mL. The addition of S9 decreased the mutagenic response, and genotoxic effects were observed at 3000-7500 µg/mL. Cytotoxic concentrations in the Ames fluctuation assay were 2500 and 10,000 µg/mL without and with microsomal activation, respectively. In the newt micronucleus test, TCA induced a small increase in the frequency of micronucleated erythrocytes at 80 µg/mL.

DeMarini *et al*. (1994) evaluated the genotoxicity of TCA using the Microscreen prophage-induction assay in *Escherichia coli* concentrations ranging from 0 to 10,000 µg/mL, with and without S9 activation. TCA did not induce a mutagenic response under either condition. In a closed-system bacterial reversion assay, TA100 was used, which contains the base-substitution allele *his*G46. This allele is the only one in the Ames *Salmonella* strains that has detected mutagenic activity of trichloroethylene and its metabolites. In this test system, TCA was not mutagenic up to cytotoxic concentrations (600 ppm without S9, and ~80 ppm with S9).

The potential of TCA to induce mutations in L5178Y/TK$^{+/-}$ –3.7.2C mouse lymphoma cells was examined by Harrington-Brock *et al*. (1998). The mouse lymphoma cells were incubated in culture medium treated with TCA concentrations up to 2150 µg/mL without S9 metabolic activation and up to 3400 µg/mL with S9. In the absence of S9, TCA increased the mutant frequency by 2-fold or greater only at concentrations resulting in ≤11% survival (2000 µg/mL or higher), leading the authors to characterize the mutagenicity of TCA as equivocal. In the presence of S9, a doubling of mutant frequency was seen at concentrations of 2250 µg/mL and higher, including several concentrations with survival >10%. No statistical evaluation of

these data was conducted. Although both small-colony and large-colony mutants were observed, no cytogenetic analysis was conducted due to the weak mutagenic response. The small-colony mutants are indicative of chromosomal damage, which cannot be attributed to low pH, as the authors stated that no pH change was observed in the presence of S9. The authors noted that TCA is one of the least potent mutagens evaluated in this *in vitro* system, and that the weight-of-evidence suggests that TCA is unlikely to be mutagenic. Other mutagenicity/genotoxicity studies support this conclusion.

Two related studies were conducted to evaluate the relationship between TCA-induced lipid peroxidation and oxidative DNA damage (Austin *et al*., 1996; Parrish *et al*., 1996), as described in detail in Section V.A. (Short-term Health Effects). In the acute study by Austin *et al*. (1996), male B6C3F1 mice (6/group) were treated with a single oral dose of TCA (0, 30, 100, or 300 mg/kg) and 8-hydroxydeoxyguanosine (8-OHdG) adducts were measured in liver DNA. A significant increase of about one-third in 8-OHdG levels was observed in the 300-mg/kg group at 8-10 hours post-dosing. Parrish *et al*. (1996) expanded on this study by evaluating TCA-induced oxidative DNA damage following repeated dosing. Male B6C3F1 mice (6/group) were exposed to 0, 100, 500, or 2000 mg/L TCA in drinking water for either 3 or 10 weeks (approximate doses of 0, 25, 125, or 500 mg/kg/day). The levels of 8-OHdG levels were unchanged at both time periods. Thus, oxidative damage to genomic DNA as measured by 8-OHdG adducts did not occur with prolonged TCA treatment.

Mackay *et al*. (1995) investigated the ability of TCA to induce chromosomal DNA damage. In an *in vitro* assay, treatment with TCA as free acid, with and without metabolic activation, induced chromosome damage in cultured human peripheral lymphocytes only at concentrations (2000 and 3500 μg/mL) that significantly reduced the pH of the medium. Neutralized TCA had no effect in this assay even at a cytotoxic concentration of 5000 μg/mL, suggesting that reduced pH was responsible for the TCA-induced clastogenicity. The authors also tested neutralized TCA in the *in vivo* bone-marrow micronucleus assay in mice. C57BL mice were given TCA intraperitoneally at doses of 0, 337, 675, or 1080 mg/kg/day for males and 0, 405, 810, or 1300 mg/kg/day for females for two consecutive days, and bone-marrow samples were collected 6 and 24 hours after the last dose. The administered doses represented 25, 50, and 80% of the median lethal dose, respectively. No treatment-related increase in micronucleated polychromatic erythrocytes was observed. To further evaluate the role of pH changes in the induction of chromosome damage, isolated liver-cell nuclei from B6C3F1 mice were suspended in a buffer at various pH levels and were stained with chromatin-reactive (fluorescein isothiocyanate) and DNA-reactive (propidium iodide) fluorescent dyes. Chromatin staining intensity decreased with decreasing pH, suggesting that pH changes alone can alter chromatin conformation. Thus, the authors conclude that TCA-induced pH changes are likely to be responsible for the chromosome damage induced by non-neutralized TCA.

Taken together, these data suggest that TCA is at most only weakly genotoxic. Although some assays have reported positive responses, the magnitude of the response has been generally reported as minimal. No mutagenicity was reported in *S. typhimurium* strain TA100 in the

absence of metabolic activation (Rapson *et al*., 1980) or in an alternative protocol using a closed system (DeMarini *et al*., 1994), but a mutagenic response was induced in this same strain in the Ames fluctuation test reported by Giller *et al*. (1997). On the other hand, mutagenicity in mouse lymphoma cells was only induced at cytotoxic concentrations (Harrington-Brock *et al*., 1998). Measures of DNA-repair responses in bacterial systems have been similarly inconclusive, with induction of DNA repair reported in *S. typhimurium* by Ono *et al*. (1991), but not by Giller *et al*. (1997) in *E. coli*. TCA induced oxidative DNA damage in the livers of mice following a single dose (Austin *et al*., 1996), but not following repeated dosing over 3 or 10 weeks (Parrish *et al*., 1996). TCA-induced DNA strand breaks and chromosome damage have been observed in several studies (Bhunya and Behera, 1987; Nelson and Bull, 1988; Giller *et al*., 1997), and were suggested by the results of Harrington-Brock *et al*. (1998), although these effects have not been uniformly reported (Chang *et al*., 1991). Recent evidence suggests that TCA-induced clastogenicity is secondary to pH changes and not a direct effect of TCA (Mackay *et al*., 1995).

E. Carcinogenicity

In studies by Herren-Freund *et al.* (1987) and Bull *et al.* (1990), TCA was shown to induce liver tumors in both male and female mice, but not in rats. The carcinogenicity of TCA has been further explored by several investigators as described below.

Principal Studies:

DeAngelo *et al*. (1997) studied the carcinogenic potential of TCA in male F344 rats. As described previously in Section V.B. (Longer-Term Effects), groups of 50 rats were administered TCA in drinking water at concentrations of 0, 50, 500, or 5000 mg/L (corresponding to time-weighted average daily doses of 0, 3.6, 32.5, and 364 mg/kg) for 104 weeks, beginning at 28-30 days of age. The maximum tolerated dose (MTD) was considered to have been reached, as indicated by a 10.7% decrease in the final body weight of high-dose animals relative to controls. Interim sacrifices were conducted at 15, 30, 45, and 60 weeks, and gross lesions in the body and internal organs were examined; the survivors were sacrificed at 104 weeks. The authors sacrificed 18-21 rats/group at the interim sacrifices. Survival rates at final sacrifice were 79%, 75%, 71%, and 86% in control, low-, mid-, and high-dose groups, respectively. Complete necropsy and histopathology examination of the liver and other tissues showed no dose-related increases in neoplasms or hyperplasia. The sensitivity of the assay is limited, however, by the relatively small group sizes for a cancer bioassay. Due to the interim sacrifices, only ~30 animals/group were exposed for more than 60 weeks. Peroxisome proliferation as measured by cyanide-insensitive palmitoyl CoA oxidase (PCO) activity in the livers of animals exposed to 364 mg/kg/day of TCA was increased about 2-fold throughout the exposure period. The other doses did not alter PCO activity. There was no evidence of an exposure-related increase in hepatocyte proliferation.

Pereira (1996) evaluated the liver carcinogenicity of TCA in female B6C3F1 mice. The mice were administered 2.0, 6.67, or 20.0 mmol/L TCA (0, 327, 1090, or 3268 mg/L) in drinking

water from 7-8 weeks of age to sacrifice following either 360 days (51 weeks) or 576 days (82 weeks) of exposure. The control group of 134 mice were administered 20 mmol NaCl, and there were 93, 46, and 38 mice in the low-, mid-, and high-dose groups, respectively. Daily doses were not reported by the study authors, but can be estimated at 0, 78, 262, and 784 mg/kg/day, based on the default drinking-water value for female B6C3F1 mice (U.S. EPA, 1988).

A significant increase in the percentage of animals with hepatocellular carcinomas was seen in the 784 mg/kg/day dose group after 51 weeks. A significant increase in the incidence of foci and hepatocellular carcinomas was induced by 262 mg/kg/day TCA after 82 weeks, and the incidence of foci, hepatocellular adenomas, and hepatocellular carcinomas was increased in the high-dose group (784 mg/kg/day) at this time point. After 51 weeks, 25% of animals treated with 784 mg/kg/day exhibited carcinomas, compared to none in the mid- and low-dose groups. After 82 weeks of treatment, statistically significant increases in altered foci were observed at 262 mg/kg/day (33.3%) and at 784 mg/kg/day (61.1%), compared to 11.1% in the controls. The incidence of adenomas and carcinomas was also increased at 82 weeks; adenomas were observed in 38.9% of the high-dose animals and carcinomas were observed in 18.5% and 27.8% of mid-dose and high-dose animals, respectively. By contrast, 2.2% of the controls had an adenoma and 2.2% of the controls had a carcinoma.

In this same study (Pereira, 1996), the characteristics of the lesions were studied to evaluate differences in mode of action of DCA and TCA. Unlike altered hepatic foci (AHF) and tumors induced by DCA, which were reported as being predominantly eosinophilic, hepatic tumors induced by TCA were predominantly basophilic or mixed basophilic and eosinophilic, and basophilic tumors, including all observed hepatocellular carcinomas (N = 11) lacked glutathione-S-transferase-pi (GSTπ) expression. Tumors in control mice were also mostly basophilic or mixed basophilic and eosinophilic. Since comparable numbers of the foci of TCA-treated animals were basophilic and eosinophilic, the author suggested that the basophilic foci induced by TCA treatment may be more likely to progress to tumors. The author also evaluated cell proliferation following 5, 12, or 33 days of treatment with TCA. TCA increased the 5-(bromo-2-deoxyuridine) BrdU-labeling index after 5 days of exposure, but not after the longer exposure durations; the degree of increase was similar for all three of the doses tested. The authors found that the tumorigenic activity of TCA was linearly related to the concentration in drinking water. Based on differences in the shape of the tumor dose-response curve and staining characteristics of tumors, the author concluded that DCA and TCA act through different mechanisms. The characteristics of the foci and tumors induced by TCA were described as being consistent with the predominant basophilic staining observed in tumors induced by peroxisome proliferators, suggesting that this pathway might be involved in the observed hepatocarcinogenicity of TCA.

In contrast to the findings of Pereira (1996), the results of a study by Carter *et al.* (2003) did not find the altered hepatic foci (AHF) and tumors induced by DCA to be predominantly eosinophilic. The reasons for the discrepancies in the findings of Pereira (1996) and Carter *et al.*

(2003) are unclear, but may be related to the size of AHFs examined, time-dependent changes in phenotypic characteristics, experimental differences in design and/or analysis, or other factors.

Von Tungeln *et al.* (2002) studied the ability of TCA to induce liver tumors in neonatal B6C3F1 mice. Male and female mice (12/sex/dose) were given total doses of 1000 or 2000 nmol TCA. For the lower dose, the animals were given i.p. injections at 8 days of age (1/3 of the total dose) and 15 days of age (2/3 of the total dose). The high-dose animals were given injections of 3/7 of the total dose on day 8 and 4/7 on day 15. High-dose animals were terminated at 12 months; low-dose animals were kept for 20 months. The treated animals did not exhibit a significant increase in the incidence of liver tumors compared to controls administered DMSO. Among the TCA exposed mice, 4 males each (17%) at the high and low doses exhibited adenomas and one male (4%) at the low dose exhibited carcinomas. No tumors were noted in females at either dose or in the control animals of either sex.

The authors also examined DNA samples from the liver for the presence of adducts that are associated with free radicals and lipid peroxidation. They found that 8-OHdG adducts in liver DNA from the TCA exposed male mice at 2000 nmol were significantly increased at 24 hours, 48 hours, and 7 days following the dose administered at 15 days of age. Malondialdehyde-associated guanine adducts were reported to be increased in liver DNA from male mice treated with 2000 nmol TCA at 24 and 48 hours following the last dose, but not at the 7 day time point.

In addition to TCA, the authors tested a variety of chemicals with the potential to generate free radicals. They concluded that their results indicated that neonatal mice were not uniquely sensitive to chemicals that generate oxidative stress. They speculated that the absence of liver carcinogenicity in the test system employed could have been the results of the low doses used and the dosing regime (dosing on days 8 and 15). They also hypothesized that any increase in cell replication induced by TCA was small in comparison with the cell proliferation occurring during early postnatal liver development minimizing TCA's activity as an indirect acting carcinogen during this life stage.

Other Studies:

Ferreira-Gonzalez *et al*. (1995) studied the K- and H-*ras* proto-oncogene mutation patterns in TCA-induced tumors in male B6C3F1 mice. The *ras* gene encodes a plasma membrane-bound GTPase. This GTPase activates kinase cascades that regulate cell proliferation. The *ras* gene was studied because changes in the rate and spectrum of mutations in the *ras* proto-oncogene have been linked to the carcinogenic mechanism of various liver carcinogens. Mice (number per group not reported) were exposed to 0 or 4500 mg/L (1080 mg/kg/day based on default water intake values in U.S. EPA, 1988) TCA in drinking water for 104 weeks. The incidence of liver carcinomas was 19% in the untreated mice and 73.3% in the TCA-exposed group. DNA samples were extracted from 32 spontaneous liver tumors from the control group, and 11 from liver tumors in mice treated with TCA. DNA samples containing

point mutations in exons 1, 2, and 3 of the K- and H-*ras* genes were detected by the presence of single-stranded conformation polymorphisms (SSCP). The SSCP analysis involved amplification of DNA from the control or tumor tissue to generate DNA fragments containing normal or mutated *ras* gene fragments. In the spontaneous tumors from control mice, *ras* mutations were detected only at the H-61 codon (i.e., the mutation was in the H-*ras* gene, in the 61st codon, which is in the second exon); 58% of the spontaneous liver carcinomas showed mutations in H-61, compared with 45% of the tumors from TCA-treated mice. One TCA-induced tumor showed a mutation in K-61 (i.e., in the K-*ras* gene, in the second exon). Comparative sequence analysis of exon 2 mutations from spontaneous and TCA-induced tumors revealed that mutations detected in the TCA tumors matched the mutation spectrum seen in the spontaneous tumors from control mice. Therefore, TCA changed neither the rate of *ras* mutations, nor the type of mutations occurring at codon 61.

Based on the absence of an effect on mutation rate, the authors indicated that it was not clear if TCA was acting through a genotoxic or non-genotoxic mechanism. The number of spontaneously-occurring tumors in control animals had a slightly higher rate of *ras* mutations than did tumors in TCA-treated animals, suggesting that TCA was likely acting through a non-genotoxic mechanism. Because TCA increased the tumor yield, but did not change mutations in *ras*, the study authors suggested that TCA might facilitate the growth of preneoplastic lesions that arise from spontaneously initiated (i.e., *ras* mutated) hepatocytes. The authors further suggested that TCA was not enhancing growth of preneoplastic lesions through increased cell proliferation, since TCA has not been demonstrated to be mitogenic, a conclusion the authors based on the results of DeAngelo *et al*. (1989). More recent studies seem to confirm this finding. Although TCA might induce hepatocyte proliferation following short-term dosing in mice (Dees and Travis, 1994; Stauber and Bull, 1997), chronic exposure of mice to TCA decreased normal hepatocyte proliferation and the high proliferation rate in altered hepatic foci was not TCA-dependent (Stauber and Bull, 1997, as presented below). As an alternative to increased cell-growth signaling to explain enhanced growth of pre-initiated cells, the authors of the current study (Ferreira-Gonzalez *et al*.,1995) suggested that TCA might be blocking pathways that suppress cell growth, such as intercellular communication. Another possible non-genotoxic mechanism might be mediated by increased peroxisomal proliferation which, based on current knowledge of other peroxisomal proliferators, has a depressing effect on apoptosis that might facilitate the growth of initiated cells (Stauber and Bull, 1997).

In a cell proliferation study by Stauber and Bull (1997), male B6C3F1 mice were pretreated with 2000 mg/L of TCA (480 mg/kg/day based on default water-intake values U.S. EPA, 1988) in drinking water for 50 weeks. The mice were then given drinking water containing 0, 20, 100, 500, 1000 or 2000 mg/L TCA (estimated doses of 0, 5, 23, 115, 230 and 460 mg/kg/day, based on default water intake values (U.S. EPA, 1988) for two additional weeks to assess whether cell proliferation induced by TCA in either normal liver cells or tumors was dependent on continued treatment. All dose groups contained 12 animals, except for the 2000 mg/L group, which consisted of 22 mice. Five days prior to sacrifice, DNA in replicating hepatocytes was labeled *in vivo* using BrdU administered via subcutaneously-implanted pumps.

Liver tissue was stained and dividing nuclei were counted. Cell division rates were evaluated separately in normal hepatocytes, in tumors, and in altered hepatic foci. A transient but significant elevation in normal hepatocyte division rates was evident in mice consuming 2000 mg/L TCA for 14 or 28 days (apparently as part of the pretreatment phase), but continued treatment for 52 weeks resulted in a significant decrease in hepatocyte division rate. In the mice treated for 50 weeks with 2000 mg/L and then shifted to other concentrations for two weeks, the cell division rate in normal liver cells was elevated (but not statistically significantly so) at 100 and 500 mg/L, but in mice exposed to 1000 or 2000 mg/L for two weeks, there was a significant decrease in cell division.

Cell division rates in TCA-induced altered hepatic foci (AHF) and tumors were high at all doses. Rates in AHF and tumors remained high in mice whose exposure was terminated during the last two weeks of the study, indicating that these rates were independent of continued TCA treatment. TCA-induced lesions were histochemically stained with anti-c-JUN and anti-c-FOS antibodies, component proteins of the AP-1 transcription factor that up-regulates expression of genes required for DNA synthesis. No differences were observed in the levels of proteins reacting with c-JUN and c-FOS antibodies in either liver AHF or tumors, relative to normal hepatocytes, indicating that TCA produces little, if any, direct stimulation of the replication of initiated cells. However, three tumors induced by TCA each contained a nodule that stained heavily for c-FOS, and cell-division rates within these nodules were very high, suggesting a transition to an aggressive tumor. The low frequency of this marker (3/52 tumors) suggested that its presence in these nodules was not due to a direct effect of TCA.

Based on these results, the study authors proposed a mechanism for TCA-induced hepatocarcinogenesis. They proposed that the initial growth stimulation induced by TCA causes normal cells to compensate by increasing signals that inhibit cell proliferation, which ultimately results in the TCA-induced growth inhibition observed with chronic treatment. Pre-initiated cells refractory to this growth inhibition would then have a selective growth advantage. The authors noted that the lack of effect on c-JUN by TCA is consistent with tumor characteristics of other peroxisome proliferators. Since cell replication in altered hepatic foci was independent of TCA, (i.e., discontinued TCA treatment did not alter AHF or tumor-cell labeling), the authors proposed that TCA might enhance growth of initiated cells by suppressing apoptosis, as has been demonstrated for other peroxisome proliferators, and consistent with peroxisome proliferation playing an important role in TCA-induced carcinogenesis.

In a study by Pereira and Phelps (1996) to assess liver tumor promotion activity by TCA, female B6C3F1 mice were treated with 25 mg/kg of the tumor initiator methylnitrosourea (MNU) at 15 days of age or given 4 mL/kg sterile saline (vehicle control). Starting at 7 weeks of age, animals were administered neutralized TCA in drinking water at concentrations of 0, 2.0, 6.67, or 20.0 mmol/L (0, 327, 1090, or 3268 mg/L) for either 31 weeks (n=8-15/group) or 52 weeks (n=39 for MNU controls, 40 for the low-dose TCA-only group, 19 for the mid- and high-dose TCA-only groups, and 6-23 for TCA + MNU groups). Dose estimates were not reported by the study authors, but the drinking-water concentrations would result in doses of approximately

0, 78, 262, and 784 mg/kg/day based on the default drinking-water value for female B6C3F1 mice (U.S. EPA, 1988). A recovery group (n=11) was removed from treatment after 31 weeks and retained for an additional 21 weeks. At 31 weeks, treated animals exhibited a slight, dose-related linear increase in relative liver weights. At 31 and 52 weeks, no significant increase in foci of altered hepatocytes, adenomas, or carcinomas was observed in mice that only received MNU.

In mice administered TCA but not initiated with MNU, the only tumorigenic response was a slight increase in the yield of hepatocellular carcinomas/animal (0.50 tumors/mouse) in the highest-dose group (784 mg/kg/day) after 52 weeks of treatment. Animals initiated with MNU and treated with TCA exhibited an increase in liver tumors following both 31 and 52 weeks of exposure in the 784 mg/kg/day group and following 52 weeks of exposure in the 262 mg/kg/day group. Both the numbers of adenomas/mouse and carcinomas/mouse were statistically elevated as compared with controls, and the tumor yield generally increased with increasing duration of exposure from 31 to 52 weeks. However, there was no significant increase in the yield of altered hepatocyte foci at either time point in any dose group. The concentration-response relationships for total lesions/mouse (foci plus tumors) after both 31 and 52 weeks of treatment were best described by a linear-regression line. When exposure to 784 mg/kg/day TCA was terminated after 31 weeks and the animals held for an additional 21 weeks, the yield of tumors/mouse remained stable.

The average yield of hepatocellular carcinomas increased from 0.20/mouse in mice exposed for 31 weeks (and held), to 0.73/mouse in mice exposed for 52 weeks. When treatment continued between weeks 31 and 52, the total mean number of tumors/mouse rose from 1.50 at 31 weeks to 4.21. These findings indicate that, although the occurrence of additional TCA-promoted tumors was dependent on continuous treatment, the stability and progression to carcinoma appeared to be independent of further treatment.

Histochemical staining indicated that more than 71% of tumors promoted with either 262 or 784 mg/kg/day TCA were basophilic and did not contain glutathione-S-transferase-pi (GSTπ), except for very small areas comprising less than 5% of the tumor. The basophilic nature of the tumors and foci promoted by TCA is consistent with the character of lesions induced by tumorigenic compounds that are rodent peroxisomal proliferators; “spontaneous” liver tumors in mice have also been reported to be predominantly basophilic and lacking GSTπ (Pereira and Phelps, 1996).

Biomarkers of cell growth, differentiation, and metabolism in proliferative hepatocellular lesions promoted by TCA were investigated by Latendresse and Pereira (1997) to further determine differences in the DCA and TCA carcinogenesis. Female B6C3F1 mice were initiated with an intraperitoneal (i.p.) injection of MNU at 15 days of age and treated with TCA in drinking water at a concentration of 20 mmol/L from age 49 days to age 413 days. The authors did not provide a dose estimate, but the approximate dose is 784 mg/kg/day, based on the default drinking water intake value for female B6C3F1 mice (U.S. EPA, 1988). At 413 days of age, the

mice were sacrificed and liver tissues were examined histologically. A panel of histochemical markers was evaluated, including: TGFα (a transforming growth factor that stimulates cell proliferation and is expressed in tumor cells), TGF-β (a transforming growth factor that is inhibitory to hepatocyte proliferation), c-JUN and c-FOS (component proteins of the AP-1 transcription factor that regulates expression of genes involved in DNA synthesis), c-MYC (a regulator of gene transcription induced during cell proliferation), the cytochrome P450s CYP2E1 (potentially involved in TCA metabolism) and CYP4A1 (induced by peroxisome proliferation signaling), and GSTπ (a phase II conjugation enzyme highly expressed in some tumor types). TCA-induced foci of altered hepatocytes and tumors tended to be predominantly basophilic, and stained variably for the histochemical markers examined. In TCA-treated mice, none of the markers stained positive in more than 50% of the cells/tumor, except c-JUN, which was observed in greater than 50% of cells from 9 of the 13 tumors evaluated. This profile of marker expression contrasts with the tumors from DCA-treated mice, for which more than half of the examined tumors expressed TGF-α, c-MYC, CYP2E1, CYP4A1, and GSTπ in greater than 50% of the cells. The contrasting histochemical-marker profiles, induced by DCA and TCA, provide evidence for a different mode of action for these two haloacetic acids.

In the case of the TCA-promoted tumors, the minimal immunostaining for most markers (with the exception of c-JUN) suggested that these proteins are not particularly important in TCA-induced tumor promotion. On the other hand, the study authors pointed out that the regional staining variability within the lesions for c-JUN and c-MYC proteins is consistent with localized clonal expansion and/or tumor progression. Non-tumor hepatocytes in TCA-treated animals were generally negative for TGF-β and GSTπ staining, and positive for CYP2E1 (centrilobular region) and CYP4A1 (panlobular region). The expression of CYP4A1 in normal hepatocytes in TCA-treated animals is consistent with TCA-induced peroxisome proliferation. However, CYP4A1 was not highly expressed in the tumor cells. This result suggests that, if peroxisome proliferation is involved in TCA-induced cancer, it is likely that the effect occurs earlier in the tumorigenic process than was evaluated in this study.

Bull and coworkers (2002) published an investigation on the contribution of TCA in trichloroethylene-induced liver cancer in B6C3F1 mice. In this investigation, three separate experiments were conducted in order to determine the differential response of the rodents to trichloroethylene (TCE) and its metabolites, TCA and DCA. The responses to TCE and DCA alone will not be discussed here except in relation to the responses induced by TCA. Male mice were administered 0, 0.5 or 2 g/L TCA or 0, 0.1, 0.5 or 2 g/L DCA for 52 weeks; or were given a combination of DCA and TCA in drinking water [0.1 or 0.5 g/L DCA given in conjunction with either dose of TCA] for the same time period. The daily doses were not calculated by the study authors but correspond to 125 and 500 mg TCA/kg/day for 0.5 and 2 g/L doses, respectively, and 25 and 125 mg DCA/kg/day for 0.1 and 0.5 g/L doses, respectively (using 0.25 L/kg/day ingestion rates for male mice; US EPA, 1988).

At sacrifice, the body and liver weights were determined, tumor incidence, tumor number, and mean tumor diameter were recorded. The combined DCA/TCA dosing regimen (2

g/L TCA + 0.5 g/L DCA) resulted in a slight but statistically significant decrease in final body weights. Liver somatic index (percentage of body weight comprised of liver) was significantly increased by all TCA dosing regimens, including those in combination with DCA, indicating that the treatment increased liver size. Both the low and high doses of TCA resulted in significant increases in tumor incidence (9/20 at 2 g/L and 11/20 at 0.5 g/L as compared to 1/20 in the vehicle control). A second group of mice also given 2 g/L TCA for 52 weeks had an even higher tumor incidence (33/40); however the tumor incidence in the vehicle controls for this experiment was 4/12. Mice dosed with DCA and TCA mixtures had tumor incidences that were very close to additive.

Several tumors from each dose group were categorized by histopathological type. The dose of 0.5 g/L TCA induced 9 total tumors (adenomas, carcinomas, and hyperplastic nodules), while the 2 g/L concentration resulted in 13 total tumors. When DCA was added to the 0.5 g/L or 2 g/L TCA dose, total tumor number was increased compared to TCA alone; further, when the TCA dose was 0.5 g/L, the 0.5 g/L DCA dose gave an increased response (21 total tumors) compared to the 0.1 g/L dose (10 total tumors). However, at the 2.0 g/L TCA dose, increasing the DCA dose from 0.1 to 0.5 g/L had no effect on total tumor burden.

The authors also investigated the expression of the oncogene c-Jun in the tumors induced by TCA and DCA using an immunoassay. They found that both doses of TCA administered singly induced only c-Jun- tumors, while DCA induced a roughly equal mixture of c-Jun+ and c-Jun- tumors. TCA combined with DCA induced a significant majority of c-Jun- tumors, with a few ($\leq$3) of mixed immunoreactivity. The exception to this was the response of 2 g/L TCA combined with 0.5 g/L DCA which induced 1% c-Jun+, 44% c-Jun-, and 52% mixed.

Sequence analysis of the H-ras codon from tumor tissue revealed a lower frequency of mutations in the DCA tumors than in the TCA-induced tumors. It is noted however, that all mutation frequencies were less than the spontaneous H-ras mutation frequency in this mouse strain. The study authors note that the H-ras mutation frequency was also lower than that reported previously (Anna *et al.*, 1994; Ferreira-Gonzalez *et al.*, 1995) and suggest that differences in mutation detection methodologies, treatment duration, and/or dose might underlie the differences. The authors evaluated the expression of proteins involved in the MAP kinase-signaling cascade to determine if the H-ras mutations in the tumors affected downstream effectors (Mek, ras, active Erk ½ , and c-Fos) and evaluated the expression of the insulin receptor to determine whether it could distinguish between the tumors induced by different compounds. Although they reported that the insulin receptor, ras, and Erk ½ were activated/increased in tumors noted in mice treated with the chlorinated compounds, these changes were also noted in control tumors. Further, the insulin receptor was induced in most liver tumors relative to normal tissue, and thus could not be a distinguishing characteristic of treatment with the chlorinated acids. The authors interpreted the overall data to indicate that TCA and DCA at these low doses were essentially additive in their ability to induce tumors; however, the tumors produced are of mixed phenotype c-Jun+/c-Jun- whereas tumors produced by TCA alone have never been c-Jun+.

Bull and coworkers (2004) expanded on the investigation into additivity of tumor induction in an assay that investigated the tumor-promoting abilities of DCA and TCA in male B6C3F1 mice induced with a single dose of 3 mg/kg vinyl carbamate at 2 weeks of age. DCA and TCA were administered in drinking water under several different combinations, some of which included carbon tetrachloride; DCA and TCA were administered at either 0.1, 0.5, or 2 g/L (25, 125, or 500 mg/kg/day using default ingestion rates for mice). The mice were sacrificed at 18, 24, 30, or 36 weeks following initiation of treatment. Final body weights and liver weights were obtained, and the livers were sliced for gross examination, then processed for histopathology. Because over 8000 liver lesions were identified given the complexity of the study, detailed histopathology could not be performed; instead, the tumors were classified as hyperplastic nodules, adenomas, or hepatocellular carcinomas.

TCA and DCA treatments both significantly increased tumor numbers and size, with significant trends observed in both these parameters with time. Varying doses of TCA with a high fixed dose of DCA modified the number of tumors produced but did not significantly affect tumor size. The high-dose of TCA (2 g/L) increased tumor numbers to a maximum at 24 weeks of treatment, which was maintained through 36 weeks. Tumors induced by the low TCA dose (0.5 g/L) approach the maximum established with the high dose at 36 weeks. The authors noted that due to the complexity and design of the study, descriptive data from the study may not be appropriate for use in human health risk assessment. They speculated that the use of an initiator increased the relative proportion of cells that were responsive to TCA, which indicates the likelihood of response to tumor promotion by this compound in an uninitiated animal.

Tao *et al.* (1996) investigated whether liver tumors initiated by MNU and promoted by TCA exhibited loss of heterozygosity (LOH) in 4 polymorphic loci on chromosome 6. According to the authors, inactivation of one or more of the polymorphic alleles at these loci may be related to the inactivation of an, as yet, unidentified tumor-suppressor gene and may be a key event in the pathogenesis of some liver tumors. This hypothesis is supported by the results of a study by Davis *et al.* (1994), in which 20% of hepatic tumors induced by perchloroethene exhibited LOH on chromosome 6, suggesting the presence of a tumor suppressor gene at this site. In this study, fifteen-day-old female B6C3F1 mice were pretreated with 25 mg/kg MNU via i.p. injection and administered TCA in drinking water at a concentration of 20.0 mmol/L (3268 mg/L) for 52 weeks. The authors did not provide a dose estimate, but the approximate dose is 784 mg/kg/day, based on the default drinking water-intake value for female B6C3F1 mice (U.S. EPA, 1988). Thirty-seven liver tumors promoted by TCA were examined for LOH using 4 polymorphic loci on chromosome 6. Ten of 37 tumors (7/27 carcinomas and 3/10 adenomas) promoted by TCA showed evidence of LOH for at least two loci on chromosome 6. The C57BL/6J alleles at both the D6mit9 and D6mit323 loci were lost in all 10 tumors exhibiting LOH, and two of these ten tumors also lost at least one of the C3H/HeJ alleles. The observed LOH on chromosome 6 in many of the tumors suggests the presence of an unidentified tumor-suppressor gene on this chromosome. However, as the majority of tumors in TCA-treated mice did not exhibit LOH on chromosome 6, the authors concluded that other molecular activity is probably involved in the hepatocarcinogenicity of TCA.

The hypomethylation of DNA by TCA was investigated by Tao *et al*. (1998) as a nongenotoxic mechanism involved in tumor promotion and carcinogenesis. Mammalian DNA naturally contains the methylated base 5-methylcytosine (5MeC), which plays a role in regulation of gene expression and DNA imprinting (Razin and Kafri, 1994); an overall decrease in the content of 5MeC in DNA is often found in tumors and has been considered to represent an important event in the clonal expansion of premalignant cells during neoplastic progression (Counts and Goodman, 1994, 1995). In this study, female B6C3F1 mice were injected intraperitoneally with 25 mg/kg of N methyl-nitrosourea (MNU) at 15 days of age; at 6 weeks of age, TCA at a concentration of 25 mmol/L (4085 mg/L) was administered in drinking water for 44 weeks. This concentration corresponds to approximately 980 mg/kg/day based on default water intake for female B6C3F1 mice in a chronic study (U.S. EPA, 1988). Control mice received only MNU. To test the effects of short-term treatment with TCA on DNA methylation, mice were given 0 or 25 mmol/L TCA, corresponding to approximately 1062 mg/kg/day, based on the strain-specific water intake for a short-term study (U.S. EPA, 1988). DNA extracted from liver tissue and tumors was hydrolyzed, and 5-methylcytosine (5MeC) and the four DNA bases were separated and quantified by HPLC. After 11 days of exposure to TCA (without pre-treatment with MNU), the level of 5MeC in total-liver DNA was decreased relative to untreated controls. After 44 weeks of TCA treatment, 5MeC levels were not different from controls that had received only MNU. No difference in DNA methylation was observed between the control groups in the short-term and long-term experiments. These results indicate that TCA caused only a transient decrease in DNA methylation in non-involved tissue. In TCA-promoted hepatocellular adenomas and carcinomas, the level of 5MeC in DNA was decreased 40% and 51%, respectively, as compared with either noninvolved tissue from the same animal or liver tissue from control animals given only MNU. Termination of TCA treatment 1 week prior to sacrifice did not change the levels of 5MeC in either adenomas or carcinomas. 5MeC levels in DNA from carcinomas were lower than in DNA from adenomas, suggesting that DNA methylation is further decreased with tumor progression. DNA hypomethylation tends to favor gene expression, which may drive cell-proliferation responses. Therefore, based on the change observed in the adenomas and carcinoma tissue compared to the uninvolved tissue, the authors suggested that hypomethylation of DNA, as indicated by decreased 5MeC in tumor DNA, is involved in the carcinogenic activity of TCA.

No studies on the carcinogenicity of TCA were identified for exposure by the dermal or inhalation routes.

F. Summary

In toxicity studies for TCA (Davis, 1986; Davis, 1990), high doses resulted in decreased food consumption and body-weight loss. Alterations in intermediary carbohydrate metabolism (e.g., decreased lactate levels in several tissues) have also been observed (Davis, 1990). The liver has consistently been identified as a target organ for TCA toxicity in short-term (Goldsworthy and Popp, 1987; DeAngelo *et al*., 1989; Sanchez and Bull, 1990; Laughter *et al.*,

2004) and longer-term (Bull *et al*., 1990; Mather *et al*., 1990; Bhat *et al*., 1991) studies. Peroxisome proliferation has been a primary endpoint evaluated, with mice reported to be more sensitive to this effect than rats. More recent studies have confirmed these earlier findings. TCA induced peroxisome proliferation in B6C3F1 mice exposed for 10 weeks to doses as low as 25 mg/kg/day (Parrish *et al*., 1996), while in rats exposed to TCA for up to 104 weeks (DeAngelo *et al*., 1997), peroxisome proliferation was observed at 364 mg/kg/day, but not at 32.5 mg/kg/day. Increased liver weight and significant increases in hepatocyte proliferation have been observed in short-term studies in mice at doses as low as 100 mg/kg/day (Dees and Travis, 1994), but no increase in hepatocyte proliferation was noted in rats given TCA at similar doses (DeAngelo *et al*., 1997). More clearly adverse liver-toxicity endpoints, including increased serum levels of liver enzymes (indicating leakage from cells) or histopathological evidence of necrosis, have been reported in rats, but generally only at high doses. For example, in a rat chronic drinking-water study, increased hepatocyte necrosis was observed at a dose of 364 mg/kg/day (DeAngelo *et al*., 1997).

The potential reproductive toxicity of TCA has not been adequately tested. No animal studies were identified that evaluated this endpoint. The results of an *in vitro* fertilization assay indicated that TCA might decrease fertilization (Cosby and Dukelow, 1992). The available data suggest that TCA may be a developmental toxicant. TCA administration was associated with increased resorptions, decreased implantations, and increased cardiovascular malformations at 291 mg/kg/day (Johnson *et al*., 1998); decreased maternal weight gain and fetal weights at a dose of 300 mg/kg/day (Fisher *et al*., 2001); and decreased fetal weight and length, and increased cardiovascular malformations at 330 mg/kg/day (Smith *et al*., 1989). However, these studies did not identify a NOAEL and developmental toxicity occurred at maternally toxic doses. The results of *in vitro* developmental-toxicity assays, including mouse and rat whole-embryo culture (Saillenfait *et al*., 1995; Hunter *et al*., 1996), and the non-mammalian *Xenopus* system (frog embryo teratogenesis assay) (Fort *et al*., 1993), have yielded given positive results; negative results have been obtained in the non-mammalian *Hydra* system (Fu *et al*., 1990). According to Fu *et al*. (1990), the *Hydra* assay accurately predicts the relative adult/developmental proportionality of standard (i.e., *in vivo*) developmental toxicity assays in over 90% of cases; discrepancies to date have all been false positives.

Negative results were reported for TCA in the Ames assay in strain TA100 in the absence of metabolic activation (Rapson *et al*., 1980); the compound was also negative in both the absence and presence of S9 in TA100, TA98, and RSJ100 (Kargalioglu *et al.*, 2002). In modified Ames assays, mixed results were reported (Giller *et al*., 1997; DeMarini *et al*., 1994), and only weakly-positive mutagenicity was reported in a mouse lymphoma cell assay (Harrington-Brock *et al*., 1998). Reports of DNA-strand breaks (Nelson and Bull, 1988; Chang *et al*., 1991) have also produced mixed results. A study by Mackay *et al.* (1995) found that chromosome damage was not induced by TCA in the absence of pH changes (Mackay *et al*., 1995); in contrast, Harrington-Brock *et al*. (1998) found evidence of TCA clastogenicity (small colonies) in mouse lymphoma cells in the absence of pH changes.

TCA administered in drinking water has been reported to induce liver tumors in mice but not in rats (Herren-Freund *et al.*, 1987). This observation has also been reported in subsequent drinking-water studies. Pereira (1996) observed an increased incidence of hepatic adenomas in female B6C3F1 mice at doses of 262 mg/kg/day and higher. In contrast, no increase in neoplastic liver lesions were found in F344 rats given doses up to 364 mg/kg/day (DeAngelo *et al*., 1997). In addition, a variety of mechanistic studies have observed that TCA induced or promoted liver tumors in mice (Ferreira-Gonzalez *et al*., 1995; Pereira and Phelps, 1996; Tao *et al*., 1996; Latendresse and Pereira, 1997; Stauber and Bull, 1997; Tao *et al*., 1998; Bull *et al.*, 2004).

No animals studies were identified on the potential systemic toxicity of TCA following dermal or inhalation exposures. However, concentrated solutions of TCA applied topically are corrosive to the skin (Eriksson *et al*., 1994).

Chapter VI. Health Effects in Humans

Most of the human health data for chlorinated acetic acids appear as components of complex mixtures of water disinfectant by-products. These complex mixtures of disinfectant by-products have been associated with increased potential for bladder, rectal, and colon cancer in humans (reviewed by Mills *et al*., 1998 and Boorman *et al*., 1999) and adverse effects on reproduction (reviewed by Mills *et al*., 1998 and Nieuwenhuijsen *et al*., 2000).

Most of the studies of human health effects following exposure to water disinfectant by-products have used total trihalomethanes as the exposure metric, and the risks attributable to chlorinated acetic acids typically have not been reported. In one study by Klotz and Pyrch (1999), a population-based case-control study was conducted on the relationship between drinking water exposure to trihalomethanes, haloacetonitriles, and haloacetic acids and neural tube defects. The study included 112 eligible cases of neural tube defects in 1993 and 1994 that were identified through the New Jersey Birth Defect and Fetal Death Registries. A total of 248 controls were selected randomly from all New Jersey births with approximately ten controls selected for each month over 24 months. No significant relationship between total trihalomethanes and neural tube defects was observed for analysis of all cases, cases restricted to subjects with known residency at conception, or cases restricted to isolated cases of neural tube defects. However, a statistically significant difference between cases and controls was observed when cases were restricted to subjects with known residency at conception and to cases with isolated neural tube defects. Based on this more stringent case definition, a prevalence odds ratio (POR) of 2.1 was reported (95% confidence interval 1.1 - 4.0) for the highest tertile (third) of trihalomethane exposure. However, only a slight non-statistically significant excess risk (POR 1.2, 95% confidence interval 0.5-2.6) was found for cases when analyzed based on total haloacetic acids tertiles. The specific haloacetic acids that were measured as part of the total haloacetic acid exposure estimate were not reported. Based on the results of the study, the authors concluded that the haloacetic acids did not exhibit a clear association with neural tube defects.

No human epidemiology studies were located for TCA. In addition to studies of disinfectant by-product mixtures as described above, TCA levels have also been evaluated in cancer epidemiology studies for humans exposed to drinking water contaminated with chlorinated solvents. Vartiainen *et al*. (1993) investigated drinking water exposures to trichloroethylene (TCE) and tetrachloroethylene (PCE) in drinking water in two villages in Finland whose drinking water was contaminated with trichloroethylene (at concentrations up to 212 μg/L) and/or tetrachloroethylene (at concentrations up to 180 μg/L). TCA, a metabolite of these chlorinated solvents, was assessed in urine samples from 87 and 21 inhabitants who consumed contaminated drinking water in the two villages, respectively. Inhabitants who did not drink the contaminated water were excluded from the analysis. Control groups included 45 volunteers from a nearby town who consumed uncontaminated ground water (ground-water control), and 15 unexposed volunteers from another city who consumed uncontaminated filtered surface water (surface-water control). Blood levels of TCA were not measured for any of the

groups. The excreted TCA doses differed significantly among the groups. Average excretion was 310 and 120 ng/kg/day in the two exposed groups, 31 ng/kg/day in the ground water controls, and 63 ng/kg/day in the surface water controls. TCA excretion in both of the exposed groups was significantly greater ($p<0.001$) than either control group, demonstrating that exposures to TCE- and PCE-contaminated drinking water results in increased internal doses of TCA. Although internal exposures of TCA were higher in the exposed groups, no corresponding increase in the rates in expected versus observed incidences of liver cancer, non-Hodgkin's lymphoma, Hodgkin's disease, multiple myeloma, or leukemia were observed in either village. The authors noted that although the study is of ecologic design, all cancer cases since 1953 were recorded in the files of the Finnish Cancer Registry, allowing a high degree of certainty in their findings.

Identified case reports demonstrate the corrosive potential of TCA to human skin. Depending upon concentration and duration of contact, TCA can denature and precipitate protein. This characteristic has been used clinically in chemical skin peeling treatments for many years. TCA at concentrations ranging from 16.9% to 50% have been used in skin peeling treatments (Rubin, 1995; Chiarello *et al*., 1996; Moy *et al*., 1996; Tse *et al*., 1996; Witheiler *et al.*, 1996; Kang *et al*., 1998). The skin peeling procedure results in a pink erythema and swelling for the first few days post-operation and is followed by exfoliation of the dead skin. Histological studies (Moy *et al*., 1996; Tse *et al*., 1996) indicate that the TCA-induced skin damage is characterized by epidermal loss, early inflammatory response, and collagen degeneration.

Nunns and Mandal (1996) reported two cases of inflammation of the vulva caused by the use of TCA in topical treatment of genital warts. In both cases, vulval and vestibular warts were treated with TCA. The surface of each wart was coated with TCA (concentration was not reported). Initially the patients complained of burning, which was short-lived. After a second TCA treatment a week later, the patients reported continual soreness or burning. On clinical examination, marked erythema and tenderness in the vulvar vestibule area was noted. The symptoms in these patients lasted for 2 to 15 weeks.

No new clinical or case studies of the effects of oral or inhalation exposure of humans to TCA were located.

Chapter VII. Mechanisms of Toxicity

A. Non-Cancer Effects

Target organs for the toxicity of TCA in humans have not been specifically identified. However, TCA induces systemic, noncancer effects in animals that can be grouped into three categories: metabolic alterations, liver toxicity, and developmental toxicity. Metabolic alterations relate to observed changes in carbohydrate metabolism in TCA-treated animals, with decreases reported in plasma glucose and lactate levels and liver lactate levels (Davis, 1990). These effects are consistent with activation of pyruvate dehydrogenase activity (reviewed in U.S. EPA, 1994).

The primary site of TCA-induced toxicity in animals studies is the liver. Collective analysis of the available studies reveals a common spectrum of liver effects that includes changes in lipid and carbohydrate homeostasis, increased liver weight, increased hepatic DNA labeling, and hepatocyte necrosis. The cellular mechanisms involved in changes in lipid and carbohydrate homeostasis have not been conclusively identified. TCA is likely to alter carbohydrate homeostasis in the liver by affecting pyruvate dehydrogenase activity (U.S. EPA, 1994). TCA is also a peroxisome proliferator (U.S. EPA, 1994; Austin *et al*., 1995; Austin *et al*., 1996; Parrish *et al*., 1996; reviewed in Bull, 2000). Activation of the peroxisome proliferation pathway induces the transcription of genes that encode enzymes responsible for fatty acid metabolism (Lapinskas and Corton, 1999), suggesting that lipid and carbohydrate homeostasis might also be affected through this mechanism.

One commonly observed histopathological change associated with perturbations in lipid and carbohydrate homeostasis is glycogen accumulation in the liver. Acharya *et al*. (1995) reported decreased levels of liver triglycerides and liver cholesterol, and increased liver glycogen in rats given TCA for 10 weeks, although relative liver weights did not differ from those of control animals. The enzymatic basis for increased hepatic glycogen accumulation remains unclear.

Although TCA-induced glycogen accumulation has not been well-studied, analogy to the same effect seen with DCA can be informative. DCA-induced glycogen accumulation can become pathological, because chronic treatment might result in glycogen stores becoming difficult to mobilize (Kato-Weinstein *et al*., 1998). The mechanism for glycogen accumulation is not known, but it may be associated with inhibition of glycogenolysis, because the observed effects resemble those observed in glycogen storage disease an inherited deficiency or alteration in one or more of the enzymes involved in glycogen metabolism.

Increased liver weight is typically observed concurrently with or at lower doses than other endpoints following oral dosing with TCA. Changes in liver weight can reflect increases in cell size, cell number, or both. TCA appears to induce both hepatocellular enlargement

(Mather *et al*., 1990; Acharya *et al*., 1997) and cell proliferation as assessed by differences in hepatocyte DNA labeling (Sanchez and Bull, 1990; Dees and Travis, 1994). Increased cell proliferation in normal cells may be transient, however, with no change or even decreased growth observed after chronic exposure (Pereira, 1996; DeAngelo *et al*, 1997). Both cytomegaly and increased cell proliferation might be explained by TCA-induced peroxisome proliferation (Lapinskas and Corton, 1999). There is little evidence that increased cell proliferation is secondary to hepatocyte cytotoxicity, as discussed below in the Cancer Mechanisms section, although TCA can induce hepatic necrosis at high doses (U.S. EPA, 1994; DeAngelo *et al*., 1997).

TCA induces developmental toxicity in rats (Smith *et al*., 1989; Fisher *et al.*, 2001; Johnson *et al*., 1998) with embryolethality (increased resorptions) and cardiovascular system malformations reported. However, developmental toxicity in these studies occurred at maternally toxic doses. Although *in vitro* test systems are limited in their utility to predict adverse developmental effects and associated toxic potencies in intact organisms, they are useful in generating mechanistic hypotheses. Whole embryo cultures have been used to assess the potential for developmental toxicity of TCA (Hunter *et al.*, 1996; Saillenfait *et al.*, 1995). TCA induces a variety of morphological changes in mouse and rat whole embryo cultures, supporting the appearance of soft-tissue malformations observed *in vivo* at maternally toxic doses. The mechanism(s) for developmental toxicity is not known.

O'Flaherty *et al*. (1992) developed a physiologically-based pharmacokinetic model for weak acids, which suggested that these substances accumulate to a greater extent in the embryo/fetal compartment than in the mother, based on the pKa of the acid and the pH gradient between the maternal plasma and the embryo compartments. TCA behaving as an acid might induce developmental toxicity by changing the intracellular pH (O'Flaherty *et al*., 1992)

A.1. Developmental Toxicity of Trichloroacetic Acid

The results of several rodent whole embryo testing studies provide mechanistic support for the potential for developmental toxicity of a number of haloacetic acids *in vivo* and suggest possible mechanisms of embryotoxicity. However, *in vitro* studies such as whole embryo culture have limited utility for predicting either the spectrum of adverse developmental effects or the associated toxic potencies in intact organisms. In addition to maternal influences in the whole animal during gestation and lactation, potentially adverse developmental responses observed *in vitro* can be modified by hepatic metabolism, toxicokinetics, the activity of additional protein systems, and other physiologic and biochemical processes. Further, the chemical concentrations required to induce developmental effects in *in vitro* experimental systems such as whole embryo culture are usually much higher than low-dose environmental exposures. Thus, these *in vitro* data are hypothesis-generating only, and must be supplemented by mechanistic data from studies conducted *in vivo.* To date, the data from *in vivo* and *in vitro* developmental toxicity studies are

limited and do not provide significant information on possible or likely mechanisms of developmental toxicity for TCA.

Hunter *et al*. (1996) examined the relative potencies of a series of haloacetic acids by calculating and comparing compound-specific benchmark concentrations, BMC_5 (i.e., the lower 95% confidence interval of the concentration that produced a 5% increase in neural tube defects) in whole mouse embryos in culture. The BMC_5 value for TCA estimated from a figure in the paper was about 1500 μM. Generally, monosubstituted acids were more potent than the di- or tri-substituted. The authors concluded that all haloacetic acids are potential developmental toxicants.

The potential developmental toxicity of TCA was also studied *in vitro* using a rat whole embryo culture system by Saillenfait *et al*. (1995). TCA induced statistically significant, concentration-related decreases in the growth and development parameters of conceptuses beginning at 1 mM.

Richard and Hunter (1996) developed quantitative structure-activity relationships (QSARs) for a range of haloacetic acids using information on similarities in structure and adverse developmental effects among related chemicals and the quantitative toxicity data set derived by Hunter *et al*. (1996) from mouse whole embryo culture testing. A QSAR was developed for 10 haloacetic acids using a regression model. This QSAR suggested that there was a common mechanism of action for haloacetic acids' developmental toxicity, implying additivity of adverse effects for haloacetic acid mixtures.

Hunter *et al*. (1999), in a published abstract, evaluated the ability of known haloacetic acid metabolites to induce dysmorphogenesis in the mouse whole embryo culture system. The potency of glycolate, glyoxylate, and oxalate were tested. Glycolate induced a low incidence of neural tube defects (NTDs) at 1000 μM, while no effects were induced by glyoxylate or oxalate at this concentration. For all three compounds the severity of effects increased with increasing concentration. The concentrations of MCA at which dysmorphogenesis was observed in the same test system were much lower than haloacetate metabolites, while TCA induced developmental effects at concentrations similar to those of the metabolites. This result suggests that the developmental toxicity of TCA, but not MCA, may be due to the metabolites glycolate, glyoxylate, or oxalate. In contrast to the QSAR analysis (Richard and Hunter, 1996), this more recent result suggests differences in the potential mechanisms by which MCA and TCA might induce developmental effects. However, *in vitro* studies are limited in their ability to predict *in vivo* developmental toxicity, as previously noted.

The limited animal data also make it difficult to compare *in vivo* fetal toxicity to the predicted relative potencies of TCA observed in whole-embryo cultures. For TCA, the developmental LOAELs for the animal studies identified were 291 mg/kg/day (Johnson *et al*., 1998), 300 mg/kg/day (Fisher *at al.*, 2001) and 330 mg/kg/day (Smith *et al*., 1989). In the Fisher

et al. (2001) and Johnson *et al*. (1998) studies, only a single dose of TCA was tested, and maternal toxicity was observed, as indicated by a decrease in maternal body weight gain relative to controls. Further, none of these studies identified a NOAEL, precluding identification of the NOAEL/LOAEL boundary. Therefore, these studies do not provide sufficient information to adequately evaluate either the developmental toxicity or the relative potency of TCA.

Preliminary results of *in vivo* testing provide some evidence for differences in the mode of action of haloacetic acids. Smith *et al*. (1992), in a published abstract, reported on interactions in the developmental toxicity of DCA and TCA. Pregnant Long-Evans rats were given gavage doses of one of 16 dose combinations of DCA at 0, 140, 1400, or 1900 mg/kg/day with TCA at 0, 50, 500, or 800 mg/kg/day during gestation days 6-15. Effects observed in dams and pups were fit to regression models to evaluate the interaction effect of DCA and TCA. Based on this analysis, the authors concluded that a synergistic interaction occurred for maternal body weight from gestation day 6-9. An antagonistic effect was reported for spleen and kidney weight, and for the proportion of resorptions, heart and total fetal malformations, and affected fetuses. Significant interactions were also reported for pup weight and crown-to-rump length. Inadequate data were provided in the abstract for evaluation of the authors' conclusions. However, the reported interactions suggest that DCA and TCA may act through different mechanisms.

B. Carcinogenic Effects

Several studies have demonstrated that TCA is a liver carcinogen in mice, but not in rats (Herren-Freund *et al.*, 1987; Bull *et al.*, 1990; DeAngelo *et al.*, 1997). Subsequent studies have been conducted to elucidate the mechanisms of TCA-induced liver carcinogenesis. The mode of action of TCA-induced liver carcinogenesis has not been conclusively identified. Bull (2000) discussed the weight of evidence for alternative mechanisms of TCA-induced rodent tumorigenesis, including direct genotoxicity, peroxisome proliferation, and altered cell proliferation. Other mechanisms for TCA-induced carcinogenesis that have been investigated in recent studies include DNA hypomethylation and changes in gap-junction intercellular communication. The potential involvement of each of these modes of action will be described here briefly.

Genotoxicity:

Moore and Harrington-Brock (2000) evaluated the weight of evidence for the genotoxicity of trichloroethylene and its metabolites, including TCA. The authors concluded that it is unlikely that TCA contributes to tumor formation through a mutational mechanism. This conclusion was based on only weak evidence for mutagenicity. In addition, although evidence for chromosome damage induction is mixed, the absence of chromosome damage following treatment with neutralized TCA suggests that TCA is not clastogenic (Mackay *et al*., 1995). Ferreira-Gonzalez *et al*. (1995) showed that the mutation frequency and mutation

spectrum in the H-*ras* gene were similar in tumors from control and TCA-treated mice, suggesting that TCA was not inducing tumors through direct DNA damage. Bull (2000) noted that benign tumors regressed after suspension of TCA treatment, and suggested that these data support a growth promotion mode of action, rather than tumor initiation events resulting from genotoxicity. Oxidative DNA damage, as measured by increases in the DNA adduct, 8-OHdG, has been evaluated as a potential mechanism of genotoxicity. However, increased oxidative DNA damage was observed after acute doses, but not following sustained exposures (Austin *et al*, 1996; Parrish *et al*., 1996), suggesting either effective DNA repair and/or adaptation to repeated TCA exposures. Taken together, the arguments described by Moore and Harrington-Brock (2000) and Bull (2000) are consistent with the existing data and suggest that TCA is not acting through a genotoxic mechanism.

Peroxisome proliferation:

TCA is a peroxisome proliferator, but whether peroxisome proliferation is the cause of TCA-induced liver tumorigenicity has not been conclusively demonstrated. In support of a causal relationship, many peroxisome proliferators are rodent liver tumorigens (reviewed in Lapinskas and Corton, 1999) and tumorigenic doses of TCA are similar to doses that induce peroxisome proliferation (Bull, 2000). The recent work of Laughter and coworkers (2004) with PPAR α-null mice indicated that the receptor was instrumental in mediating the increased liver to body weight ratios, hepatocyte proliferation, gene expression, and increased lipid metabolism enzymes in TCE-exposed mice, but these effects were not consistently observed in TCA-exposed wild-type mice. Further, while TCA induces peroxisome proliferation in both mice and rats, it is only tumorigenic in mice. Bull (2000) noted that, under similar dosing regimens, a 2- to 3-fold increase in peroxisome proliferation was observed in F344 rats compared to a 10-fold increase over controls in mice (strains not specified), although this relationship may not hold for all mouse and rat species and strains. For example, Bull further noted that Wistar rats displayed a higher induction of peroxisome proliferation than mice. The lack of tumorigenicity in F344 rats (DeAngelo *et al*., 1997) could reflect a lower affinity of the peroxisome proliferation pathway for TCA, which would result in a smaller peroxisome response in rats as compared to mice. If mice are indeed more sensitive to peroxisome proliferation, this line of reasoning would argue that peroxisome proliferation might be causally related to TCA-induced rodent tumors, with the absence of tumors in rats reflecting the inability to induce a sufficiently robust peroxisome response to lead to tumor formation. However, the role of peroxisome proliferation in selectively favoring the growth of initiated cells is not well-characterized, and there may be other mechanisms associated with this proposed mode of action.

Further, the relevance of increased peroxisomal proliferation to the development of tumors in humans is believed to be either low or non-existent. Humans have been reported to be less affected by exposure to peroxisomal proliferators than either mice or rats (Lapinskas and Corton, 1999; Bentley *et al*., 1993). A recent *in vitro* study by Walgren *et al*. (2000) supports this conclusion. Human primary hepatocyte cultures derived from several donors were used to

study the effects of TCA on peroxisomal activity, as measured by palmitoyl-CoA oxidation activity, and DNA synthesis, as measured by the rate of hepatocyte incorporation of radioactivity from radiolabeled thymidine. A potent peroxisome proliferator, WY-14,643 [4-chloro-6-(2,3-xylidino)-2-pyrimidinylthio] acetic acid, was used as the positive control. Cultured human hepatocytes exhibited a limited peroxisomal proliferative response to both TCA and WY-14,643. Induction of peroxisomal enzyme activity was not observed with either compound. DNA synthesis decreased following treatment with either TCA or WY-14,643. The response of the human cell cultures to TCA was highly variable, attributed by the authors to result from variation in expression or regulation of signaling molecules affected by TCA exposure. The authors concluded that these studies demonstrate that human cells are much less sensitive than rodent cells to treatment with peroxisome proliferators, including TCA. However, as previously noted, it is not yet clear whether peroxisome proliferation is a key event in the development of TCA-induced mouse hepatocarcinogenesis.

Altered cell proliferation:

TCA-induced changes in cell growth regulation have also been suggested as a mechanism for the formation of liver tumors. There is little evidence that hepatocyte cytotoxicity followed by regenerative hyperplasia is involved. As described above for noncarcinogenic liver effects of TCA, increased liver weight is consistently reported as a low-dose effect in numerous studies, but liver necrosis is generally either not reported or occurs only at much higher doses (reviewed in U.S. EPA, 1994; Dees and Travis, 1994; Parrish *et al*., 1996; Acharya *et al*., 1995; Pereira; 1996). In addition, hepatocyte hypertrophy and hyperplasia are commonly induced by peroxisome proliferators (Lapinskas and Corton, 1999).

TCA has been shown to increase hepatocyte proliferation in DNA-labeling experiments (U.S. EPA, 1994; Dees and Travis, 1994). Dees and Travis (1994) observed increased hepatic DNA labeling at doses lower than those associated with evidence of necrosis, suggesting that TCA-induced cell proliferation is not due to regenerative hyperplasia. The authors reached this conclusion based on (1) the pattern of observed histopathological changes, which indicated nodular areas of cellular proliferation, and (2) the results of liver DNA labeling experiments, which showed incorporation of [^{3}H]thymidine in extracted liver DNA, but no difference in total liver DNA content (mg DNA/g liver). The authors concluded that their results are consistent with an increase in DNA synthesis and cell division in response to TCA treatment. The authors further suggested that the absence of histopathological effects makes it unlikely that the increased radiolabel was secondary to tissue repair.

Investigations of the effects of TCA on cell growth rates have produced conflicting results. Miyagawa *et al*. (1995) examined the effect of TCA (and a battery of putative nongenotoxic liver carcinogens and noncarcinogens) on replicative DNA synthesis (RDS), to assess the utility of measurement of cell proliferation as a screening assay for detecting nongenotoxic carcinogens. Groups of male B6C3F1 mice (four or five per dose) were

administered a single oral gavage dose of TCA in an acute toxicity test to determine the maximally tolerated dose (MTD). The MTD for TCA was 500 mg/kg, determined as approximately one-half of the LD_{50}. Groups of 4 or 5 animals were administered a single oral gavage dose of one-half of the MTD (250 mg/kg) or the MTD (500 mg/kg) and incorporation of [^{3}H]thymidine in harvested hepatocytes was measured 24, 39, or 48 hours after dosing. For TCA, positive responses were observed at 250 mg/kg at 24 and 39 hours (6.5- and 4.9-fold above controls), and 500 mg/kg (9.8-fold above controls). Although the mean increase in RDS met the criteria for a positive response, the increases did not appear to be statistically significant, based on the standard deviations supplied in the summary table.

In contrast to the increased cell proliferation observed by Miyagawa *et al*. (1995), Channel and Hancock (1993) found that TCA can decrease the rate of progression through S-phase of the cell cycle. WB344 cells, a non-tumorigenic epithelial rat hepatocyte cell line, were exposed to untreated medium or medium containing 100 μg/mL TCA. Cell growth rates were assessed by cell counting, and transition through the cell cycle was monitored by labeling nascent DNA with BrdU. The resulting labeling data were used to identify fractions of cells in various stages of the cell cycle and to model transit times through each phase. The transit time through S-phase was estimated to be 5.20 hours for treated and 5.02 hours for control cells, respectively ($p<0.05$). As further support for this effect, cells in S-phase were elevated by approximately 5-20% for the first 6 hours after release from TCA-treatment, but returned to control values after this initial period. In contrast to these results indicating slowing of S-phase transit, relative movement plots (also related to S-phase transit time) did not differ from controls. The authors suggested, however, that this might reflect the insensitivity of relative movement plots for detection of small treatment-related changes, such as those observed for TCA. The authors suggested that the observed pattern of cell cycle perturbation, a slightly extended period of S-phase, would be consistent with a sublethal effect of cytotoxicity and would be less serious than a decrease in transit time through G_2M phase (which could potentially increase chromosomal mismatches and rearrangements, due to an insufficient time spent in mitosis). The importance of these results by Miyagawa *et al*. (1995) and Channel and Hancock (1993) are difficult to interpret, as they might not reflect the cell growth conditions of normal hepatocytes *in vivo*. For this reason, these studies are of limited use in evaluating the effects of TCA on cell growth *in vivo*, but are summarized here for completeness.

In vitro studies also support the conclusion that TCA does not induce tumors through cell growth secondary to necrosis, because TCA does not appear to be highly toxic to hepatocytes. Pravecek *et al*. (1996) evaluated the hepatotoxicity of DCA and TCA in liver slices from male B6C3F1 mice and the metabolic capacity of the liver for these two compounds. In the cytotoxicity studies, the liver slices were exposed for up to 8 hours at concentrations of TCA ranging from 0 to 86 mM. Cytotoxicity was dependent on the duration of exposure, with a greater effect observed at 8 hours than at 3 or 6 hours. Estimated EC_{50} concentrations were reported for each of four measures of cytotoxicity, including potassium leakage, lactate dehydrogenase activity (LDH), AST, and ALT activities in the medium. Estimated EC_{50} values

ranged from 64 to 72 mM for potassium leakage, LDH activity, and AST activity, while no dose-response was observed for ALT activity. In another *in vitro* study using hepatocyte suspensions from male $B6C3F_1$ mice and Sprague-Dawley rats, the possible role of cytotoxic effects in contributing to TCA-induced hepatocarcinogenicity was evaluated (Bruschi and Bull, 1993). Cytotoxicity was measured by the release of lactate dehydrogenase and by trypan-blue exclusion in the exposed cells, as well as by depletion of intracellular reduced glutathione. No effects were seen in TCA-treated cells at concentrations up to 5.0 mM and exposure times up to 240 minutes, suggesting that little cytotoxicity occurs from exposure to TCA as measured by the biomarkers employed. Thus, the *in vitro* results suggest that TCA is not highly cytotoxic to hepatocytes.

Rather than regenerative hyperplasia, differential effects on growth of normal and initiated cells has been suggested as a mode of action of TCA. Bull (2000) suggested that TCA acts by increasing the clonal expansion of initiated cells, while decreasing growth of normal cells. Data from Stauber and Bull (1997) were cited as evidence for this mode of action. In this experiment, mice were exposed to a high concentration of TCA for 50 weeks, and then removed from treatment or continued at the same exposure for an additional 2 weeks. Evaluation of cell proliferation found that the growth of TCA-initiated tumor cells was high, and similar levels were seen in mice removed from TCA treatment and in animals maintained on TCA for the entire experiment. By contrast, replication was inhibited in normal hepatocytes. Thus, initiated cells would have a growth advantage over growth-inhibited normal cells following continuous treatment.

Bull (2000) argued that TCA might not only inhibit growth of normal cells, but may also enhance growth of initiated cells with certain phenotypes, based on the results of Stauber *et al*. (1998). Stauber *et al*. (1998) demonstrated that TCA increases cell proliferation of c-JUN negative hepatocytes *in vitro*. These investigators treated isolated hepatocytes from neonatal mice with TCA at concentrations ranging from 0 to 2.0 mM, and plated the cells to allow them to form colonies. Exposure of the cells to 0.5 mM TCA and above significantly increased colony formation in the absence of cytotoxicity, as compared with controls. Anchorage-independent colonies were induced by TCA in a dose-dependent manner and were c-JUN negative, which is the same phenotype observed in TCA-induced liver tumors in mice exposed *in vivo* to TCA. The expression of c-JUN was not induced when isolated hepatocytes were cultured as monolayers in the presence of 2.0 mM TCA, indicating that TCA selectively affects subpopulations of anchorage-independent hepatocytes. The authors concluded that the results of this study demonstrated that TCA promotes the survival and growth of different populations of initiated hepatocytes.

The ability of TCA to act as a tumor promoter (Pereira and Phelps, 1996; Latendresse and Pereira, 1997) supports the selective growth mode of action described in Bull (2000). The ability of a variety of peroxisome proliferators to induce cell proliferation (Lapinskas and Corton, 1999) is also consistent with this proposed mode of action, (i.e., enhancement of the selective growth of initiated cells), and has been proposed as a possible mechanism for

tumorigenesis. It is not yet known whether changes in cell division might be directly caused by peroxisome proliferation or whether peroxisome proliferators might activate initiated cell growth pathways parallel to peroxisome signaling (Lapinskas and Corton, 1999). The absence of a tumorigenic response of the peroxisome proliferator WY-14,643 in mice having targeted disruption of the peroxisome proliferation receptor α (PPARα) (Peters *et al*., 1997) provides some evidence that the tumorigenicity of peroxisome proliferators might be directly related to downstream signaling events linked to receptor activation. If TCA-induced tumors are dependent on the activation of the peroxisome pathway, then humans would be expected to be less sensitive than rodents to TCA-induced tumorigenesis, because humans are believed to have a much lower response to peroxisome proliferators (Lapinkas and Corton, 1999: Bentley *et al*., 1993). However, the role of peroxisome proliferation in selectively favoring the growth of initiated cells is not well-characterized, and there may be other mechanisms associated with this proposed mode of action.

Other mechanisms:

In addition to peroxisome signaling pathways, changes in cell growth regulation through DNA methylation changes or altered cell-cell communication have been explored. Tao *et al*. (1998) reported that hypomethylation, as indicated by decreased 5-methyl-cytosine (5MeC) in tumor DNA, might be involved in the promotion of tumors by facilitating aberrant gene expression (Counts and Goodman, 1995). In female B6C3F1 mice initiated by an i.p. injection of MNU, and then administered TCA in drinking water at 25 mM for 44 weeks, the level of 5MeC in DNA of hepatocellular adenomas and carcinomas was decreased 40% and 51%, respectively, as compared with noninvolved tissue from the same animal and control animals given only MNU; termination of TCA treatment 1 week prior to sacrifice did not change the levels of 5MeC in either adenomas or carcinomas.

In a more recent study on DNA methylation and cell proliferation in B6C3F1 mice (Ge *et al*., 2001), female B6C3F1 mice treated with daily gavage doses of 500 mg/kg TCA and sacrificed at 24, 36, 48, 72, and 96 hours after the first dose, showed a significant increase in the proliferating cell nuclear antigen (PCNA)-labeling index in liver cells at 72 and 96 hours relative to controls. The mitotic index was also elevated at 96 hours. Assessment of the tumor promoter region of *c-myc* proto-oncogene in the liver, by Southern blot analysis, indicated that DNA in treated animals showed hypomethylation of the internal cytosine of CCGG sites in this region, beginning between 48 and 72 hours following initiation of TCA treatment, and increasing between 72 and 96 hours. TCA also decreased methylation in the promoter region of *c-myc* gene in the kidney and urinary bladder after 72 and 96 hours, but not at earlier times, after commencement of treatment. Hypomethylation was greater in the liver than in the kidney or urinary bladder. The authors proposed that TCA-induced hypomethylation was responsible for the observed increase in DNA replication (evidenced by increased PCNA index and mitotic index), and that this epigenetic activity was mechanistically associated with TCA carcinogenicity.

The laboratory continued investigating the role of hypomethylation in DNA, in particular of the insulin-like growth factor II (IGF-II) gene (Tao *et al.*, 2004). Tissue from liver tumors and from tumor free areas of the liver were taken from female B6C3F1 mice induced with a single injection of MNU and given drinking water containing 20 mM TCA for 46 weeks (reported previously in Pereira and Phelps, 1996). The authors found that TCA decreased 5MeC levels significantly in liver tumor DNA and to a lesser extent in non-involved tumor DNA. Further, methylation was decreased at several CpG sites in the upstream region of the differentially methylated region-2 (DMR-2) of the IGF-II gene. mRNA expression of the IGF-II gene was increased 5.1-fold in TCA-induced tumor tissue, but not in the non-involved liver tissue from TCA-exposed mice. The authors suggested these results support the hypothesis that DNA hypomethylation is involved in the mechanism for TCA liver carcinogenesis.

Effects of TCA on intercellular communication have also been studied. Clone 9 (ATCC CRL 1439), a normal liver epithelial cell line from a 4-week old Sprague-Dawley male rat, was used to assess the effects of TCA on gap junction intercellular communication (IC) (Benane *et al*., 1996). The cells were grown in a nutrient mixture, plated, and exposed to TCA at a range of concentrations for varying time periods. Lucifer yellow scrape-load dye transfer was used as a measure of IC. Following an initial screen to identify the lowest concentration at which TCA affected dye transfer, the main study was conducted at concentrations of 0, 0.5, 1.0, 2.5, and 5 mM. Cells were treated for 1, 4, 6, 24, 48, or 168 hours. At a concentration of 0.5 mM, there were no statistically significant differences in IC between control and treated cells at any of the time points. At a concentration of 1.0 mM, statistically significant differences were found for all time periods except 4 and 168 hours. At concentrations of 2.5 and 5 mM, the level of dye transfer was statistically decreased as compared with controls for all time points. The lowest concentration and shortest time to reduce dye transfer was 1 mM over a 1-hour period. The reduction in IC increased with higher concentrations and longer treatment time. TPA, a tumor promoter used as positive control, caused a rapid reduction in IC. From a physiological perspective, the formation of gap junctions with short half-lives in cell membranes can be considered a regulatory control factor and contribute to altered cellular growth and differentiation patterns (Benane *et al*., 1996).

Summary:

In summary, TCA is clearly carcinogenic in mice (U.S. EPA, 1994; Pereira, 1996; Bull *et al.*, 2002). Numerous recent studies have investigated the mechanism by which TCA induces liver tumors. The data do not support a direct genotoxic mechanism (Bull, 2000; Moore and Harrington-Brock, 2000). Rather, tumor induction appears to involve perturbation of cell growth, both through growth inhibition of normal cells and proliferation of selected cell populations (Stauber and Bull, 1997; Stauber *et al*., 1998; Bull, 2000). Mechanisms of altered cell growth control that have been evaluated include activation of the peroxisome signaling pathway (U.S. EPA, 1994; Austin *et. al*, 1996; Parrish *et al*., 1996; Bull, 2000), global DNA hypomethylation (Tao *et al*., 1998), and reduced intracellular communication (Benane *et al*.,

1996). However, the existing evidence is not sufficiently developed to determine which, if any, of these mechanisms are causally related to the observed tumor responses.

C. Sensitive Subpopulations

Age-dependent differences in susceptibility to TCA have not been tested in systemic toxicity studies. The dose spacing in the available developmental-toxicity studies is inadequate to determine the relative fetal and maternal toxicity of TCA. The LOAELs for developmental toxicity are 291 mg/kg/day (Johnson *et al*., 1998), 300 mg/kg/day (Fisher *et al*., 2001), and 330 mg/kg/day (Smith *et al*., 1989). However, these LOAELs occurred at maternally toxic doses. These developmental-toxicity data are too limited to draw any conclusions on whether the developing organism might be a sensitive subpopulation. In subchronic toxicity studies, a LOAEL and NOAEL of 355 and 36.5 mg/kg/day, respectively, was observed in male rats exposed to TCA in drinking water for 90 days (Mather *et al*., 1990). In the Parrish *et al*. (1996) 10-week drinking water study with male mice, the LOAEL and NOAEL were 125 and 50 mg/kg/day, respectively.

These data suggest, but do not show conclusively, that systemic effects are observed at dose levels similar to, or less than, those at which developmental toxicity occurs. Therefore, it is likely that regulatory values based on systemic toxicity will be protective of developmentally toxic effects.

The data are also insufficient to determine whether there are age-dependent differences in the metabolism of the haloacetic acids that might lead to differences in health risk. The enzymes responsible for the metabolism of TCA have not been conclusively identified. The health implications of any differences between children and adults in metabolic capacity are also difficult to determine for the haloacetic acids, since the toxic form of each compound has not been identified. The mechanisms involved in haloacetic acid toxicity are not sufficiently developed to make this determination. The preliminary results of Hunter *et al*. (1996) in whole embryo culture suggest that, at least for the developmental effects, the parent compound may be involved in the toxicity of MCA, while for TCA a metabolite may be involved. However, *in vitro* studies such as whole embryo culture have limited utility for predicting the developmental toxicity of chemical agents in intact organisms and are considered to be useful only for hypothesis-generation, not for hypothesis-testing. Further *in vivo* studies are needed to determine whether there are age-related differences in TCA susceptibility.

D. Interactions

Acharya *et al*. (1995; 1997) evaluated liver and kidney toxicity of TCA as part of a study on the interactions in the toxicity of tertiary butyl alcohol and TCA. Young male Wistar rats (5-6/dose) were exposed for 10 weeks to untreated drinking water or drinking water containing 25 ppm (3.8 mg/kg/day) TCA, 0.5% tertiary-butyl (t-butyl) alcohol (740 mg/kg/day), or both. Estimated doses were calculated based on the strain-specific default body weight and water intake. Combined treatment with t-butyl alcohol and TCA increased the effect over that seen with either chemical alone for increased liver weight, liver glycogen accumulation, and serum glucose. Although not quantified by the study authors, these effects were modest and generally reflected an additive or less than additive effect with the co-treatment. Combined treatment had no affect over single treatments for terminal body weight, serum levels of liver enzymes, liver triglycerides, or liver or kidney glutathione levels. Both tertiary butyl alcohol and TCA induced histopathological changes in the liver and kidney, when administered singly. No interactions in the spectrum or magnitude of histopathological changes were observed following combined treatment with these two compounds.

Haloacetic acid mixtures

The promoting effects of mixtures of DCA and TCA were examined by Pereira *et al*. (1997). Female B6C3F1 mice were initiated at the age of 15 days with 25 mg/kg *N*–methyl-–nitrosourea. Subsequently from 6 to 50 weeks of age, the mice were administered in their drinking water either DCA at concentrations of 7.8, 15.6, or 25 mM (1006, 2011, or 3224 mg/L, equivalent to 241, 483, and 773 mg/kg/day based on the strain-specific default water intake, U.S. EPA, 1988) with or without 6.0 mM TCA (980 mg/L, equivalent to 235 mg/kg/day based on the strain-specific default water intake, U.S. EPA, 1988). Other mice received TCA at concentrations of 6.0 or 25 mM (980 or 4085 mg/L, equivalent to 235 and 980 mg/kg/day based on the strain-specific default water intake, U.S. EPA, 1988) with or without 15.6 mM DCA (483 mg/kg/day). At the higher TCA concentration, there was a significant increase in relative liver weight. TCA at 25 mM but not at 6.0 mM significantly increased the yield/mouse of both total proliferative lesions and adenomas in a linear manner, with or without the addition of 15.6 mM DCA. At the higher TCA concentration of 25.0 mM the addition of 15.6 mM DCA resulted in a more than additive increase in the number of altered hepatocyte foci (0.31 and 0.52 per mouse for TCA and DCA alone, respectively, 2.63 per mouse when administered as a mixture) and total proliferative lesions (0.82 and 0.84 per mouse for TCA and DCA alone, respectively, versus 3.16 per mouse when administered as a mixture). Thus, a mixture at these concentrations produced more than an additive increase in the yield of altered hepatocyte foci and total proliferative lesions.

Histochemical staining of the lesions in this study revealed that all altered hepatocyte foci and adenomas were basophilic in mice treated with TCA alone and eosinophilic in DCA-treated mice. However, foci in mice receiving DCA with TCA at either concentration were

eosinophilic. Eosinophilic foci and lesions were consistently positive for the presence of GSTπ, whereas basophilic lesions lacked GSTπ, irrespective of the treatment. The only exception was in the treatment group receiving 25 mM TCA alone, in which four carcinomas were predominantly basophilic but contained small areas of GSTπ positive hepatocytes. The authors concluded that the basophilic nature and absence of GSTπ in TCA-promoted foci and tumors suggest that its tumor promoting activity is similar to that of other peroxisome proliferators. The synergistic increase in the yield of altered hepatocyte foci and lesions induced by mixtures of DCA and TCA resulted in lesions that exhibited characteristics of DCA-promoted lesions and were inconsistent with the activity of other peroxisome proliferators. However, a recent study by Carter *et al.* (2003) has demonstrated that several types of DCA-induced liver lesions, including small and large altered hepatic foci, adenomas, and carcinomas, tend to be basophilic rather than eosinophilic; therefore, these findings are inconsistent with those reported in the Carter *et al.* (2003). The reason for these differences are unclear.

E. Summary

TCA induces systemic, noncancer effects in animals and humans that can be grouped into three categories: metabolic alterations, liver toxicity; and developmental toxicity. The primary site of TCA toxicity in animals is the liver (U.S. EPA, 1994; Dees and Travis, 1994; Acharya *et al*., 1995; Acharya *et al*., 1995; DeAngelo *et al.*, 1997). Peroxisome proliferation may play a role in at least some of the observed liver effects in rodents (U.S. EPA, 1994; Austin *et al*., 1996; Parrish *et al*, 1996; Lapinskas and Corton, 1999; Bull, 2000). However, human liver cells appear to be less sensitive than rodent liver cells to the effects of peroxisomal proliferators, suggesting that this mechanism of hepatotoxicity and/or hepatocarcinogenicity might not be relevant to humans (Walgren *et al*., 2000).

Although TCA induces developmental toxicity in rats (Smith *et al*., 1989; Johnson *et al*., 1998, Fisher *et al.*, 2001), the mechanisms for developmental toxicity are not known. *In vitro* testing has suggested that one or more metabolites of TCA, rather than parent TCA, might be responsible for its developmental toxicity; however, *in vitro* systems have limited utility in predicting adverse developmental effects in intact organisms, and further *in vivo* studies are needed.

A variety of mechanisms have been suggested as contributing to TCA-induced liver tumorigenesis. Of these, peroxisome proliferation and/or altered regulation of cell growth have been best supported. There is little evidence for a role of direct genotoxicity of TCA itself (Moore and Harrington-Brock, 2000; Mackay *et al*., 1995), oxidative DNA damage (Parrish *et al*., 1996), or regenerative hyperplasia (Pereira, 1996; DeAngelo *et al*., 1997; Bull, 2000). The role of peroxisome proliferation is not clear, as this response is activated in both mice and rats, but liver tumors are only induced in mice (U.S. EPA, 1994; Pereira, 1996; DeAngelo *et al*., 1997; Bull, 2000). A better case can be made for altered proliferation in subpopulations of cells

having selective growth advantages (Stauber *et al*., 1998), arising for example, due to spontaneous mutations (Ferreira-Gonzalez *et al*., 1995).

Chapter VIII. Quantification of Toxicological Effects

The quantification of toxicological effects of a chemical consists of separate assessments of noncarcinogenic and carcinogenic health effects. Chemicals that do not produce carcinogenic effects are believed to have a threshold dose below which no adverse, noncarcinogenic health effects occur. Carcinogens are assumed to act without a threshold unless there are data elucidating a nonmutagenic mode of action and demonstrating a threshold for the precursor events that commit a cell to an abnormal tumorigenic response.

A. Introduction to Methods

A.1. Quantification of Noncarcinogenic Effects

A.1.1. Reference Dose

In the quantification of noncarcinogenic effects, a Reference Dose (RfD) (formerly called the Acceptable Daily Intake [ADI]) is calculated. The RfD is "an estimate (with uncertainty spanning approximately an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without appreciable risk of deleterious effects over a lifetime" (U.S. EPA, 1993). The RfD is derived from a no observed adverse effect level (NOAEL), lowest observed adverse effect level (LOAEL), or a NOAEL surrogate such as a benchmark dose identified from a subchronic or chronic study, and divided by a composite uncertainty factor(s). The RfD is calculated as follows:

$$RfD = \frac{\text{NOAEL or LOAEL}}{UF}$$

where:

NOAEL = No-observed-adverse-effect level expressed in mg/kg/day from a high-quality toxicological study of an appropriate duration

LOAEL = Lowest-observed-adverse-effect level expressed in mg/kg/day from a high-quality toxicological study of an appropriate duration. In situations where there is no NOAEL for a contaminant but there is a LOAEL, the LOAEL can be used for the RfD calculation with the inclusion of an additional uncertainty factor.

UF = Uncertainty factor chosen according to EPA/NAS guidelines

Selection of the uncertainty factor to be employed in calculation of the RfD is based on professional judgment, while considering the entire database of toxicological effects for the chemical. To ensure that uncertainty factors are selected and applied in a consistent manner, the Office of Water (OW) employs a modification to the guidelines proposed by the National Academy of Sciences (NAS, 1977, 1980). According to the EPA approach (U.S. EPA, 1993), uncertainty is broken down into its components, and each dimension of uncertainty is given a quantitative rating. The total uncertainty factor is the product of the component uncertainties.

The individual components of the uncertainty are as follows:

UF_H A 1, 3, or 10-fold factor used when extrapolating from valid data in studies using long-term exposure to average healthy humans. The intermediate factor of 3 is approximately ½ $\log_{10}$ unit, i.e., the square root of 10. This factor is intended to account for the variation in sensitivity (intraspecies variation) among the members of the human population.

UF_A An additional factor of 1, 3, or 10 used when extrapolating from valid results of long-term studies on experimental animals when results of studies of human exposure are not available or are inadequate. This factor is intended to account for the uncertainty involved in extrapolating from animal data to humans (interspecies variation).

UF_S An additional factor of 1, 3, or 10 used when extrapolating from less-than-chronic results on experimental animals when there are no useful long-term human data. This factor is intended to account for the uncertainty involved in extrapolating from less-than-chronic NOAELs to chronic NOAELs.

UF_L An additional factor of 1, 3, or 10 used when deriving an RfD from a LOAEL, instead of a NOAEL. This factor is intended to account for the uncertainty involved in extrapolating from LOAELs to NOAELs.

UF_D An additional factor of 1, 3, or 10 used to adjust for the absence of data on toxicological endpoints considered critical for assessing human risk. Frequently, it is applied if data for endpoints that need to be experimentally addressed in specialized studies (e.g. reproductive and developmental toxicity) are lacking." The 3-fold factor is often used when there is a single data gap exclusive of chronic data.

In establishing the UF, it is recognized that professional scientific judgment must be used. The total product of the uncertainty factors and modifying factor should not exceed 3000. If the assignment of uncertainty results in an UF product that exceeds 3000, then the database does not support development of an RfD. The quantification of toxicological effects of a chemical consists of separate assessments of noncarcinogenic and carcinogenic health effects.

Unless otherwise specified, chemicals which do not produce carcinogenic effects are believed to have a threshold dose below which no adverse, noncarcinogenic health effects occur, while carcinogens are assumed to act without a threshold.

A.1.2. Drinking Water Equivalent Level

The drinking water equivalent (DWEL) is calculated from the RfD. The DWEL represents a drinking-water-specific lifetime exposure at which adverse, noncarcinogenic health effects are not anticipated to occur. The DWEL assumes 100% exposure from drinking water. The DWEL provides the noncarcinogenic health effects basis for establishing a drinking water standard. For ingestion data, the DWEL is derived as follows:

$$DWEL = \frac{(RfD) \times BW}{WI}$$

where:

BW = 70 kg adult body weight

WI = Drinking water intake (2 L/day)

A.1.3. Health Advisory Values

In addition to the RfD and the DWEL, EPA calculates Health Advisory (HA) values for noncancer effects. HAs are determined for lifetime exposures as well as for exposures of shorter duration (1-day, 10-day, and longer-term). The shorter-duration HA values are used as informal guidance to municipalities and other organizations when emergency spills or contamination situations occur. The lifetime HA becomes the MCLG for a chemical that is not a carcinogen.

The shorter-term HAs are calculated using an equation similar to the RfD and DWEL; however, the NOAELs or LOAELs are derived from acute or subchronic studies of a duration consistent with the HA duration and identify a sensitive noncarcinogenic endpoint of toxicity. The HAs are derived as follows:

$$HA = \frac{\text{NOAEL or LOAEL} \times BW}{UF \times WI}$$

where:

NOAEL or LOAEL = No- or lowest-observed-adverse-effect-level in mg/kg bw/day

BW = Assumed body weight of a child (10 kg) or an adult (70 kg)

UF = Uncertainty factor, in accordance with EPA or NAS/OW guidelines

WI = Assumed daily water consumption of a child (1 L/day) or an adult (2 L/day)

Using the above equation, the following drinking water HAs are developed for noncarcinogenic effects:

- 1-day HA for a 10-kg child ingesting 1 L water per day.
- 10-day HA for a 10-kg child ingesting 1 L water per day.
- Longer-term HA for a 10-kg child ingesting 1 L water per day.
- Longer-term HA for a 70-kg adult ingesting 2 L water per day.

Each of these shorter-term HA values assumes that the total exposure to the contaminant comes from drinking water.

The lifetime HA is calculated from the DWEL and takes into account exposure from sources other than drinking water. It is calculated using the following equation:

$$\text{Lifetime HA} = \text{DWEL} \times \text{RSC}$$

where:

DWEL = Drinking water equivalent level

RSC = Relative source contribution. The fraction of the total exposure allocated to drinking water.

A.2 Quantification of Carcinogenic Effects

In 1986, EPA established a five-category, alphanumeric system for carcinogen with the publication of *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1986). The five categories were as follows:

Group A: Human Carcinogen.
Group B: Probable Human Carcinogen.
Group B1: Limited evidence in humans.
Group B2: inadequate evidence in humans.
Group C: Possible Human Carcinogen.
Group D: Not classified as to Human Carcinogenicity.

Group E: Evidence of Noncarcinogenicity for Humans.

In 1996 the Agency issued proposed revisions to *Guidelines for Carcinogen Risk Assessment* for public comment. The 1996 proposal was later refined and released as a revised draft in 1999 (US EPA, 1999). Although the 1999 version of the *Guidelines for Carcinogen Risk Assessment* had not yet been formally adopted by the agency, use of the 1986 guidelines ceased in 2000 with the publication of a directive from the administrator (Federal Register, 2001) specifying that the 1999 guidelines were to be used on an interim basis.

Under the U.S. EPA *Guidelines for Carcinogen Risk Assessment*: *Draft Final*, the U.S. EPA presents the carcinogenic potential of a chemical compound in a narrative fashion, and uses one of the following five standard descriptors to express the conclusion regarding the weight of evidence for carcinogenic hazard potential:

- Carcinogenic to humans
- Likely to be carcinogenic to humans
- Suggestive evidence of carcinogenic potential, but not sufficient to assess human carcinogenic potential
- Data inadequate for an assessment of human carcinogenic potential
- Not likely to be carcinogenic to humans

Each standard descriptor is presented only in the context of a chemical-specific, weight-of-evidence narrative. Additionally, more than one conclusion may be reached for an agent (e.g., an agent is "likely to carcinogenic" by inhalation exposure and "not likely to be carcinogenic" by oral exposure.

In cases where the toxicological evidence leads to the classification of the contaminant as a carcinogen or likely to be a carcinogen, mathematical models are used to calculate the estimated excess cancer risk associated with ingestion of the contaminant in drinking water. The data input to the models usually come from lifetime-exposure studies in animals. In order to predict the risk for humans, animal doses must be converted to equivalent human doses. The conversion can include corrections for noncontinuous exposure, less-than-lifetime studies and allometric scaling of the animal body weight. The dose-response assessment is performed in two stages. A mathematical assessment of observed experimental dose data is used to derive a point of departure (POD) and the 95% confidence interval on the POD[3] dose. This is followed by extrapolation to lower exposures where necessary. Extrapolation may assume either linearity or nonlinearity of the dose-response relationship, or both. The linear approach is used for mutagenic carcinogens, i.e. those with linear mode of action, or where the mode of action cannot be determined. For carcinogens with a well-substantiated nonlinear mode of action,

[3] A "point of departure" (POD) marks the beginning of extrapolation to lower doses. The POD is an estimated dose (expressed in human-equivalent terms) near the lower end of the observed range, without significant extrapolation to lower doses (U.S. EPA, 1999).

precursor effects (the preferable situation) or tumorigenic responses are modeled using approaches comparable to the benchmark options for non-cancer effects. In both cases a range of models are available.

With the linear approach the slope of the line from the point of departure (central estimate) and from the lower 95% confidence interval on the dose are calculated. The slope factors (q_1*) are a reflection of the cancer potency. They are used to calculate the concentration in drinking water that is equivalent to a specific risk to the population. Risk estimates are generally presented for the one-in-ten thousand risk (1/10,000; E-4), the one-in-one hundred thousand (1/100,000; E-5) risk and the one-in a million (1/1,000,000; E-6) risk using the following equation:

$$\text{Drinking Water Concentration} = \frac{\text{Risk x Body Weight}}{\text{Slope Factor x 2 L/day}}$$

It is assumed that the average adult human-body weight is 70 kg and that the average water consumption of an adult human is two liters of water per day. Drinking water regulations target the E-4 to E-5 risk range as determined from the lower confidence bound on the POD.

The scientific database used to calculate and support the setting of cancer risk rates has an inherent uncertainty due to the systematic and random errors in scientific measurement. Thus, there is uncertainty when the risk is extrapolated from epidemiological or animal data to the entire humans population. When developing cancer-risk rates, some of the uncertainties that exist include incomplete knowledge concerning the health effects of contaminants in drinking water, the impact of the experimental animal's age, sex and species, the nature of the target-organ system(s) examined and the actual rate of exposure of the internal targets in experimental animals or humans. Dose-response data usually are available only for high levels of exposure, not for the lower levels of exposure at which a standard may be set. When there is exposure to more than one contaminant, additional uncertainty results from a lack of information about possible synergistic or antagonistic effects. The true risk to humans, while not identifiable, is not likely to exceed the upper limit estimate and, in fact, may be lower or even zero.

B. Noncarcinogenic Effects

Table VIII-1 summarizes the available studies on the oral toxicity of TCA.

B.1. One-Day Health Advisory for TCA

No suitable data were identified for derivation of a One-day health advisory (U.S. EPA, 1994). In the absence of a suitable study, the Ten-day health advisory of 3 mg/L is used as a conservative estimate of the One-day health advisory.

B.2 Ten-Day Health Advisory for TCA

Numerous short-duration oral-toxicity studies were identified for TCA, with liver and body weight changes commonly noted effects following gavage or drinking-water exposures for periods ranging from 7 to 30 days (U.S. EPA, 1994). Only three short-term studies were identified for consideration. Sanchez and Bull (1990) exposed male B6C3F1 mice to TCA in drinking water for 14 days. Estimated TCA doses were 0, 75, 250, or 500 mg/kg/day, based on the default water intake and body weight for male B6C3F1 mice (U.S. EPA, 1988). A dose-related increase in liver weight was observed beginning at 75 mg/kg/day, but did not reach statistical significance until 250 mg/kg/day. At 500 mg/kg/day, there was an increase in both hepatocyte diameter, which was presumed to result from glycogen accumulation, and in hepatic labeling. The NOAEL was judged to be 75 mg/kg/day for increased liver weight. Austin *et al*. (1995) identified a LOAEL of 250 mg/kg/day in male B6C3F1 mice exposed to TCA in drinking water for 14 days, based on increased liver weight supported by changes in markers of lipid peroxidation. However, only one dose level was tested and therefore, it was not possible to determine either a dose-response or a NOAEL. Parrish *et al*. (1996) treated male B6C3F1 mice with 0, 25, 125, or 500 mg/kg/day TCA in drinking water for 21 days. The LOAEL was considered to be 125 mg/kg/day, based on increases in relative liver weight and increased palmitoyl-CoA oxidase activity, a measure of peroxisome proliferation, and the NOAEL was judged to be 25 mg/kg/day.

Similar results were observed in a more recent oral-gavage study. Male and female B6C3F1 mice were administered oral-gavage doses of 0, 100, 250, 500, or 1000 mg/kg/day TCA dissolved in corn oil for 11 days (Dees and Travis, 1994). Statistically significant increases in liver weight were observed at all dose levels, but no dose-response relationship was found. Dose-dependent increases in hepatic labeling index were observed beginning at the low dose of 100 mg/kg/day in males, and beginning at 250 mg/kg/day in females. The effects at the low dose were of minimal severity and there is minimal evidence for the adversity of the observed small changes. Therefore, the low dose of 100 mg/kg/day is an equivocal LOAEL for increased liver weight and hepatocyte proliferation.

The developmental toxicity of TCA has been evaluated in three studies. Smith *et al*. (1989) reported a LOAEL of 330 mg/kg/day for increased maternal spleen and kidney weight, decreased fetal weight, decreased crown-rump length, and increased cardiac malformations in Long-Evans rats. Exposure was by gavage on gestation days 6-15. The LOAEL was the lowest dose tested and no NOAEL was identified. Johnson *et al*. (1998) exposed female rats via drinking water on gestation days 1-22 to 0 or 291 mg/kg/day TCA. The single dose tested was a developmental LOAEL, based on cardiac defects, decreased implantations, and increased

Table VIII-1. Summary of Oral Studies of TCA Toxicity

Reference	Species	Route	Exposure Duration	Endpoints	NOAEL mg/kg/day	LOAEL mg/kg/day	Comments
General Toxicity - Short-term Studies							
Dees and Travis (1994)	B6C3F1 mice (male and female)	Oral Gavage in Corn oil	11 days; 0, 100, 250, 500, or 1000 mg/kg/day	↑ liver weight; ↑ hepatocyte labeling	-	100	LOAEL was judged to be equivocal in males due to the mild severity of effects, the increase in DNA labeling was small but statistically significant; clearly adverse effects (e.g., liver histopathological changes) were only observed at the highest dose.
Austin *et al.* (1995)	B6C3F1 mice (male)	Oral Drinking Water	14 days; 0, or 250 mg/kg/day	↑ liver weight; peroxisome proliferation	-	250	Doses estimated based on default drinking water intakes for male B6C3F1 mice.
Sanchez and Bull (1990)	B6C3F1 mice (male)	Oral Drinking Water	14 days; 0, 75, 250, or 500 mg/kg/day	↑ liver weight	75	250	Doses estimated based on default drinking water intakes for male B6C3F1 mice. At 500 mg/kg/day inc. hepatocyte diameter (presumably due to glycogen accumulation) and inc. hepatic labeling were observed.
Parrish *et al.* (1996)	B6C3F1 mice (male)	Oral Drinking Water	3 or 10 wks; 0, 25, 125, 500 mg/kg/ day	↑ liver weight; peroxisome proliferation	25	125	Doses estimated based on default drinking water intakes for male B6C3F1 mice.

Table VIII-1. Summary of Oral Studies of TCA Toxicity

Reference	Species	Route	Exposure Duration	Endpoints	NOAEL mg/kg/day	LOAEL mg/kg/day	Comments
Acharya *et al.* (1995; 1997)	Wistar rats (male)	Oral Drinking Water	10 wks; 0 or 3.8 mg/kg/day	Terminal body weight, liver and kidney histopath. effects; changes in liver lipid and carbohydrate homeostasis	-	3.8	Doses estimated based on default drinking water intake values for mice. 3.8 mg/kg/day is judged as an equivocal LOAEL due to the minimal severity of the liver changes, and because the results are of low reliability due to inconsistency with other well-reported studies.
General Toxicity - Longer-term Studies							
Mather *et al.* (1990)	Sprague Dawley rats (male)	Oral Drinking Water	90 days; 0, 4.1, 36.5, or 355 mg/kg/day	↓ spleen weight; ↑ liver weight and liver histopathol. changes	36.5	355	Critical Study for 1994 Longer-term health advisory.
Pereira (1996)	B6C3F1 mice (female)	Oral Drinking Water	51 or 82 wks; 0, 78, 262, and 784 mg/kg/day	↑ liver weight	78	262	Increased liver weight was observed after 82 weeks at 262 mg/kg/day. 262 mg/kg/day was judged as an equivocal LOAEL in the absence of other liver toxicity effects. Noncancer endpoints other than body weight and liver weight were not evaluated.
DeAngelo *et al.* (1997)	F344 rats (male)	Oral Drinking Water	104 wks; 0, 3.6, 32.5, or 364 mg/kg/day	↓ body weight, ↑ serum ALT activity	32.5	364	Time-weighted average daily doses were calculated by the authors.

Table VIII-1. Summary of Oral Studies of TCA Toxicity

Reference	Species	Route	Exposure Duration	Endpoints	NOAEL mg/kg/day	LOAEL mg/kg/day	Comments
Bull *et al.* 1990	B6C3F1 mice	Oral drinking water	(a) 0, 137 or 300 mg/kg/ day for 52 wks (b) 0, 270 mg/kg/ day for 37 wks +15 wk recovery	↑ liver weight, cytomegaly, glycogen accumulation	137 for 52 weeks	270 for 37 weeks	Only the liver was evaluated. Dose estimated by the authors
Developmental Toxicity							
Johnson *et al.* (1998)	Sprague Dawley rats	Oral Drinking Water	Gestation days 1-22; 0 or 291 mg/kg/day	↑ in cardiac malformations; ↓ mean implant. sites/litter, and sig. ↑ resorption sites/litter	--	291	The dose, a maternal LOAEL, was estimated by the authors, based on the animals' mean daily water consumption. Study not adequately reported; a complete array of standard developmental end points was not assessed.
Smith *et al.* (1989)	Long-Evans rats	Oral Gavage	Gestation days 6-15; 0, 330, 800, 1200, 1800 mg/kg/day	↓ fetal weight, crown-rump length, teratogenicity (cardiac), ↑ maternal spleen and kidney wts.	–	330	Critical Study for 1994 RfD. The developmental LOAEL was also a maternal LOAEL.
Fisher *et al.* (2001)	Sprague-Dawley rats	Oral, gavage	Gestation days 6-15	↓ maternal weight gain, fetal weight	--	300	The developmental LOAEL was also a maternal LOAEL

Notes: wks, weeks; ↑, increased;↓, decreased; wts, weights; sig., significant

resorptions. The maternal LOAEL was also 291 mg/kg/day, as evidenced by a 30% decrease in maternal body weight gain among treated females. A more recent study by Fisher *et al.* (2001) identified 300 mg/kg/day as a LOAEL based on decreased maternal weight gain and decreased fetal weights (individual animal and litter basis). None of these studies identified a developmental or maternal NOAEL. Thus, they are of limited suitability for the development of a 10-day health advisory. The Johnson *et al*. (1988) and Fisher *et al.* (2001) studies used only one dose level, and it was unclear whether all standard developmental endpoints, including skeletal and internal visceral malformations, were examined. Due to these limitations, the LOAEL could be lower, but it is unlikely that a higher LOAEL would be identified in a well-designed study. The Smith *et al*. (1989) study is a gavage study, and it is preferable (although not required) to use a drinking water study, instead of a gavage study, for development of health advisories.

Of the four short-term studies that evaluated liver effects in mice, only Sanchez and Bull (1990) and Parrish *et al*. (1996) identified both a NOAEL and LOAEL. The studies of Sanchez and Bull (1990), and Parrish *et al*. (1996) both evaluated liver effects in male B6C3F1 mice using drinking-water exposures, with a range of doses for establishing a dose-response. Although both studies evaluated the effect in liver in the same species and strain, the study duration is longer in the Parrish *et al*. (1996) study (21 days vs. 14 days). Also, the NOAEL identified in Parrish *et al*. (1996) is based on an increase in liver weight, in conjunction with increased palmitoyl-CoA oxidase activity, a measure of peroxisome proliferation. Sanchez and Bull (1990) identified a higher NOAEL, 75 mg/kg/day, based only on an increase in liver weight, with a corresponding LOAEL of 250 mg/kg/day. The Parrish *et al*. (1996) study is selected for the development of Ten-day HA, because of its longer study duration and measurement of more sensitive endpoints.

Based on a NOAEL of 25 mg/kg/day for liver toxicity (increased relative liver weight and peroxisome proliferation) in mice exposed for 21 days (Parrish *et al*., 1996), the Ten-day health advisory can be calculated as shown below. The default uncertainty factor of 10 is used to account for extrapolation from an animal study, since no adequate data on mouse-to-human differences in toxicokinetics or toxicodynamics were identified. Humans are much less responsive to peroxisome proliferators than mice (Lapinskas and Corton, 1999). However, although TCA is a peroxisome proliferator, and peroxisome proliferation is associated with increased liver weight, the evidence is not sufficient to move away from the default value of 10. The role of peroxisome proliferation in TCA-induced hepatic toxicity has not been conclusively determined, and other mechanisms of toxicity might account for the observed effects.

The default uncertainty factor of 10 is used to account for human variability in the absence of data on the variability in the toxicokinetics of TCA in humans or in human susceptibility to TCA. Based on these considerations, the composite uncertainty factor is 100.

$$\text{Ten-day HA} = \frac{(25\ \text{mg/kg/day})\ (10\ \text{kg})}{(100)\ (1\ \text{L/day})} = 2.5\ \text{mg/L (rounded to 3 mg/L)}$$

where:

25 mg/kg/day	=	NOAEL for liver toxicity in mice given TCA in drinking water for 21 days (Parrish *et al*., 1996).
10 kg	=	assumed body weight of a child.
100	=	composite uncertainty factor, chosen to account for extrapolation from a NOAEL in animals, and inter-individual variability in humans.
1 L/day	=	assumed daily water consumption by a 10-kg child.

B.3 Longer-Term Health Advisory for TCA

In previously reviewed subchronic oral-dosing studies (U.S. EPA, 1994) and in more recent studies (Parrish *et al*., 1996; Acharya *et al*., 1995; Acharya *et al*., 1997) in mice or rats, the liver was commonly identified as a target for TCA. Among the older studies, only one study identified both a NOAEL and a LOAEL. Mather *et al*. (1990) exposed Sprague-Dawley rats to TCA in drinking water for 90 days. The NOAEL was 36.5 mg/kg/day and the LOAEL was 355 mg/kg/day for reduced spleen weight, increased liver weight, and liver histopathological changes including hepatocellular enlargement, intracellular swelling, and glycogen accumulation. Evidence that accumulated glycogen becomes resistant to mobilization after longer-term exposure, at least in the context of DCA-induced glycogen accumulation (Kato-Weinstein *et al*., 1998), makes this an appropriate potential endpoint for derivation of the Longer-term health advisory.

Of the newer studies, two studies were identified as candidates for the derivation of the Longer-term health advisory. Acharya *et al*. (1995; 1997) exposed male Wistar rats to TCA in drinking water for 10 weeks, resulting in an estimated dose of 3.8 mg/kg/day. They found decreased terminal body weight, altered liver lipid and carbohydrate levels, and liver and kidney histopathological changes. However, only one dose level was used in this study, precluding a determination of dose-response. Further, results from this study are considered to be of low reliability due to discrepancies between the reported critical effect levels and NOAEL and LOAEL values for several other studies. For example, in Acharya *et al*. (1995; 1997) the LOAEL was approximately 10-fold lower than the NOAEL in the Mather *et al*. (1990) rat study, following examination of a similar range of endpoints in the same species (although the strain was different) exposed by the same route. In addition, the NOAEL/LOAEL boundary for liver effects in a chronic drinking-water study in rats (DeAngelo *et al*., 1997) was very similar to the results of Mather *et al*. (1990). Taken together, the weight of the evidence suggests that the NOAEL/LOAEL boundaries identified in Mather *et al*. (1990) are more representative of the body of evidence regarding the potency of TCA as a liver toxicant in rats.

The second study is one in which Parrish *et al*. (1996) exposed male B6C3F1 mice to 0, 25, 125, and 500 mg/kg/day for 10 weeks. Body weight and liver weight were evaluated and several indicators for peroxisome proliferation were measured. Absolute and relative liver weights, as well as indicators of peroxisome proliferation, were increased at the two highest doses. This study identified a LOAEL and NOAEL in mice of 125 and 25 mg/kg/day, respectively. This mouse NOAEL of 25 mg/kg/day is similar to the rat NOAEL of 36.5 mg/kg/day in the Mather *et al*. (1990) study, although slightly lower. The liver is the target organ of toxicity in both species, and thus the results from these two studies are in general agreement. Although the mouse NOAEL (Parrish *et al*., 1996) is slightly lower than the rat NOAEL (Mather *et al*., 1990), the rat study was selected for the development of a Longer-term health advisory because (1) the rat study is more robust, in that a wide range of endpoints were measured, including organ pathology and histopathology, whereas measurements in the Parrish *et al*. (1996) study were limited to changes in body weight, liver weight, and peroxisome proliferation markers; (2) the NOAELs from both studies are similar, as previously noted, and thus the rat NOAEL (36.5 mg/kg/day) is protective against the adverse effects observed at the LOAEL (125 mg/kg/day) in the mouse study; and (3) other data, including mechanistic data, strongly suggest that the rat is the more appropriate animal model for TCA than the mouse. Further, the 90-day Mather *et al*. (1990) drinking water study in rats is of appropriate duration, with compound administration occurring by the preferred route of exposure, and demonstrates a dose-response which is consistent with the results of other studies in rats (DeAngelo *et al*., 1997) and in mice (Parrish *et al*., 1996).

As noted previously, the developmental studies by Smith *et al*. (1989), Johnson *et al*. (1998) and Fisher *et al.* (2001) are limited, and these studies are not preferred for the development of health advisories. Smith *et al*. (1989) identified a developmental LOAEL of 330 mg/kg/day, the lowest dose tested, and a NOAEL could not be determined. Further, the route of compound administration was by gavage, instead of drinking water. In the Johnson *et al.* (1998) and Fisher *et al.* (2001) studies, only one dose level was reported; developmental toxicity occurred at a maternally toxic dose, and it is not clear from the study reports whether standard guidelines for assessing developmental toxicity were followed or whether all appropriate developmental end points were evaluated.

Based on a NOAEL of 36.5 mg/kg/day for liver histopathology changes in rats exposed for 90 days (Mather *et al*., 1990), the Longer-term health advisory can be calculated as shown below. This study and NOAEL were also the basis for the Longer-term health advisory derived in the earlier criteria document (U.S. EPA, 1994). A default uncertainty factor of 10 for extrapolation from rats to humans is appropriate, based on the toxicokinetic differences between rats and humans. The evidence to date, although limited, suggests that plasma clearance of TCA may be slower in humans than in rodents (Volkel *et al*., 1998; Lash *et al*., 2000), and, therefore, for a given internal dose, humans may be exposed to more TCA for a longer duration than rodents. However, these studies measured TCA as a metabolite of administered chlorinated solvent, and it is not clear the extent to which TCA in plasma was influenced by species differences in either the internal dose of the parent compound (due to differences in systemic

absorption) or in the rate of formation of TCA metabolite. Further studies are needed to assess species differences in TCA toxicokinetics. On the other hand, toxicodynamic differences between rodents and humans might favor decreasing the value of the uncertainty factor. The mechanism by which TCA induces liver toxicity is not known, but might involve peroxisome proliferation, as described above for the Ten-day health advisory. Rodents, particularly mice, are more sensitive than humans to peroxisome proliferation (Lapinskas and Corton, 1999), and mice may be more sensitive than humans to TCA-induced hepatotoxicity. *In vitro* data also suggest that human liver cells are relatively insensitive to chemical induction of peroxisome proliferation as compared with mouse liver cells (Walgren *et al*., 2000). However, in the absence of data confirming a causal relationship between peroxisome proliferation and liver damage, it is not appropriate to conclude that animals are more sensitive to TCA, and thus no adjustment to the default animal-to-human uncertainty factor is made. The default uncertainty factor of 10 is used to account for human variability, in the absence of data on the variability in the toxicokinetics of TCA in humans or in human susceptibility to TCA.

An additional uncertainty factor of 10 is used to account for database insufficiencies. Although subchronic and chronic studies of TCA have been reported for multiple species, many studies have focused on liver lesions, and a full evaluation of all potential target organs is not available for a subchronic or chronic study in a species other than the rat. Other data gaps include the lack of a multi-generation reproductive study and the lack of a developmental toxicity study in a second species. Further, a number of *in vitro* alternative screening models, including mammalian embryo culture testing (Hunter *et al.*, 1996; Sallenfait *et al.*, 1995) and the nonmammalian FETAX assay (Fort *et al.*, 1993) suggest that TCA might be a developmental toxicant. The composite-uncertainty factor used is 1000.

$$\text{Longer-Term HA (child)} = \frac{(36.5\ \text{mg/kg/day})\ (10\ \text{kg})}{(1000)\ (1\ \text{L/day})} = 0.4\ \text{mg/L}$$

where:

36.5 mg/kg/day	=	NOAEL for liver histopathological changes observed in rats given TCA in drinking water for 90 days (Mather *et al*., 1990).
10 kg	=	assumed body weight of a child.
1000	=	composite uncertainty factor, chosen to account for extrapolation from a NOAEL in animals, inter-individual variability in humans, and insufficiencies in the database, including the lack of a full histopathology data in a second species, the lack of a developmental toxicity study in second species, and the lack of a multi-generation reproductive study.
1 L/day	=	assumed daily water consumption by a 10-kg child.

The Longer-term HA for a 70-kg adult consuming 2 L/day of water is calculated as follows:

$$\text{Longer-Term HA (adult)} = \frac{(36.5\ \text{mg/kg/day})(70\ \text{kg})}{(1000)(2\ \text{L/day})} = 1.3\ \text{mg/L (rounded to 1 mg/L)}$$

where:

36.5 mg/kg/day = NOAEL for liver histopathological changes observed in rats given TCA in drinking water for 90 days (Mather *et al*., 1990).

70 kg = assumed body weight of an adult.

1000 = composite uncertainty factor, chosen to account for extrapolation from a NOAEL in animals, inter-individual variability in humans, and insufficiencies in the database, including lack of full histopathological data in a second species, the lack of a developmental toxicity study in second species, and the lack of a multi-generation reproductive study.

2 L/day = assumed daily water consumption by a 70-kg adult.

B.4 Reference Dose and Drinking Water Equivalent Level for TCA

Two chronic oral drinking-water studies were identified as potential candidates to derive the RfD and DWEL (Pereira *et al*., 1996; DeAngelo *et al*., 1997). The study by DeAngelo *et al*. (1997) yielded a NOAEL of 32.5 mg/kg/day and a LOAEL of 364 mg/kg/day for decreased body weight, increased serum ALT activity, and liver histopathological changes. In a cancer study in mice that evaluated only a limited set of end points (Pereira, 1996), a higher NOAEL for liver effects of 78 mg/kg/day was identified. Developmental toxicity studies (Fisher *et al.*, 2001; Johnson *et al*., 1998; Smith *et al*., 1989) identified developmental LOAELs for rats in drinking water or by gavage of 291, 300 and 330 mg/kg/day, respectively but failed to identify a NOAEL. However, as previously noted, these studies are on the low end of reliability. In addition, a health advisory based on developmental effects in these studies would be no more protective than one based on systemic effects, since systemic NOAELs are comparable to 1/10 the developmental LOAELs (equivalent to using an uncertainty factor for extrapolating from a LOAEL to a NOAEL). Although the developmental study by Smith *et al*. (1989) was used to derive the RfD in the draft criteria document (U.S. EPA, 1994), the chronic bioassay by DeAngelo *et al*. (1990) is considered more appropriate at this time. The route of exposure was drinking water, a dose-response was noted, both a LOAEL and NOAEL were determined, and the data in this chronic study are consistent with the findings in both the Pereira *et al.* (1996) chronic drinking water study and the Mather *et al*. (1990) subchronic drinking water study. Further, while the Pereira *et al*. (1996) chronic drinking water study in mice has a higher

NOAEL, and more clearly delineates a NOAEL/LOAEL boundary, it only evaluated a limited number of noncancer endpoints.

The RfD is based on the NOAEL of 32.5 mg/kg/day for liver histopathological changes in rats exposed to TCA in drinking water at concentrations of 0, 50, 500, or 5000 mg/L (corresponding to time-weighted average daily doses of 0, 3.6, 32.5, and 364 mg/kg) for 104 weeks (DeAngelo et al., 1997). Although no neoplasms were reported, increased abnormalities in liver histopathology were noted at the highest dose tested, yielding a NOAEL of 32.5 mg/kg/day for this end point. A default uncertainty factor of 10 is used to account for extrapolation from an animal study, as insufficient data on rat-to-human differences in toxicokinetics or toxicodynamics were identified, as described above for the Longer-term health advisory. The default uncertainty factor of 10 is used to account for human variability in the absence of data on the variability in the toxicokinetics or toxicodynamics of TCA in humans or on differences in human susceptibility to TCA. An additional uncertainty factor of 10 is used to account for database insufficiencies. Although subchronic and chronic studies of TCA have been reported for multiple species, many studies have focused on liver lesions and a full evaluation of a wide range of potential target organs has not been conducted in two different species. Other data gaps include a multi-generation reproduction study, and a developmental toxicity study in a second species. The two developmental studies in rats identified LOAELs for developmental effects but did not identify a NOAEL making this endpoint one of possible concern. The composite-uncertainty factor used is 1000.

Step 1: Determination of the Reference Dose (RfD) for TCA.

$$\text{RfD} = \frac{(32.5 \text{ mg/kg/day})}{(1000)} = 0.0325 \text{ mg/kg/day, rounded to } 0.03 \text{ mg/kg/day}$$

where:

32.5 mg/kg/day	=	NOAEL for liver histopathological changes for rats exposed in drinking water for 2 years (DeAngelo *et al*., 1997).
1000	=	composite uncertainty factor chosen to account for extrapolation from a NOAEL in animals, inter-individual variability in humans, and insufficiencies in the database, including the lack of full histopathological data in a second species, the lack of a developmental toxicity study in second species, and the lack of a multi-generation reproductive study.

Step 2: Determination of the Drinking Water Equivalent Level (DWEL) for TCA.

$$\text{DWEL} = \frac{(0.03 \text{ mg/kg/day})\ (70 \text{ kg})}{(2 \text{ L/day})} = 1.05 \text{ mg/L}$$

where:

0.03 mg/kg/day	=	RfD (after rounding)
70 kg	=	assumed body weight of an adult.
2 L/day	=	assumed daily water consumption by a 70-kg adult.

Step 3: Determination of the Lifetime Health Advisory for TCA.

$$\text{Lifetime HA} = \frac{(1.05\ \text{mg/L})\ (20\%)}{10} = 0.021\ \text{mg/L (rounded to 0.02 mg/L)}$$

where:

1.05 mg/L	=	DWEL
20%	=	estimated relative source contribution from water (see Chapter IV)
10	=	additional safety factor based on OW policy to account for possible carcinogenicity of Group C carcinogens.

C. Carcinogenic Effects

No studies were identified on the carcinogenicity of TCA in humans. As summarized previously (U.S. EPA, 1994), TCA induces liver tumors in mice, but not in rats. More recent studies have confirmed carcinogenicity in mice (Ferreira-Gonzalez *et al*., 1995; Pereira, 1996; Pereira and Phelps, 1996; Tao *et al*., 1996; Latendresse and Pereira, 1997; Pereira *et al*., 1997) and noncarcinogenicity in rats (DeAngelo *et al*., 1997). Much of the recent data on the carcinogenicity of TCA has been geared toward an evaluation of the mode(s) of action of TCA carcinogenesis.

The existing data are consistent with a non-genotoxic mechanism, based on minimal evidence in genotoxicity studies and positive results in tumor-promotion studies. Although these data suggest that TCA selectively affects cell growth in initiated, but not uninitiated, cells (Bull, 2000), the cellular mechanisms for these differences in cell proliferation remain unclear. Understanding the relevance of peroxisome proliferation in TCA-induced responses will be important in determining the relevance of the observed mouse-liver tumors for carcinogenic risk in humans. Because humans have significantly lower responses than rodents to peroxisome proliferation (Lapinskas and Corton, 1999; Bentley *et al*., 1993; Walgren *et al*., 2000), positive demonstration that this response drives the tumorigenicity in mice would suggest that humans are at less risk than mice. However, at this time, neither the bioassay nor the mechanistic data

are sufficient to determine the potential human liver cancer risk resulting from lifetime exposure to TCA.

Similarly, in view of the conflicting results between rodent species in adequately-conducted cancer bioassays, the lack of genotoxicity of TCA in numerous studies, the uncertainty regarding the likely mode(s) of action of TCA-induced mouse hepatocarcinogenicity, and the questionable human relevance of the finding of increased liver tumors in a rodent species (mouse) with a high background rate of spontaneously-occurring liver tumors, the data are insufficient to conduct a dose-response quantification for cancer.

Following EPA's 1999 *Guidelines for Carcinogen Risk Assessment*, the toxicity data for TCA are described as having "*suggestive evidence of carcinogenicity, but not sufficient to assess human carcinogenic potential*." This descriptor is appropriate when the evidence from human or animal data is suggestive of carcinogenicity, raising a concern for carcinogenic effects, but is not sufficient for a conclusion as to human carcinogenic potential. Quantification of dose-response assessment is not recommended for chemicals with this descriptor. IARC (2004) recently determined that there were inadequate evidence in humans and limited evidence in experimental animals for the carcinogenicity of TCA and placed the compound in Group 3, "*not classifiable as to its carcinogenicity to humans*."

D. Summary

Tables VIII-2 summarize HA and DWEL values that have been derived from available toxicological dose-response data for TCA.

Table VIII-2. Summary of Development of the HAs and DWEL for TCA

Study	NOAEL/ LOAEL (mg/kg/day)	UF_H	UF_A	UF_S	UF_L	UF_D	Composite Factor	RfD Equivalent (mg/kg/day) (not rounded)	Final Value (mg/L)
One-day HA									
Parrish et al. (1996)	25/125	10	10	-	1	NA[a]	100	0.25	3[b]
Ten-day HA									
Parrish et al. (1996)	25/125	10	10	-	1	NA	100	0.25	3
Longer-Term HA									
Mather et al. (1990)	36.5/355	10	10	1	1	10	1000	0.037	0.4 (child) 1.0 (adult)
DWEL									
DeAngelo et al. (1997)	32.5/364	10	10	1	1	10	1000	0.03	1[c]

a. Database uncertainty factors are not applied in the duration of One-day or Ten-day health advisories per Office of Water policy.

b. The One-day health advisory was derived from the Ten-day health advisory.

c. A Lifetime HA of 0.02 mg/L was derived from this value, using a RSC of 20% and a safety factor of 10 to account for possible carcinogenicity of Group C carcinogens.

Chapter IX. References

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